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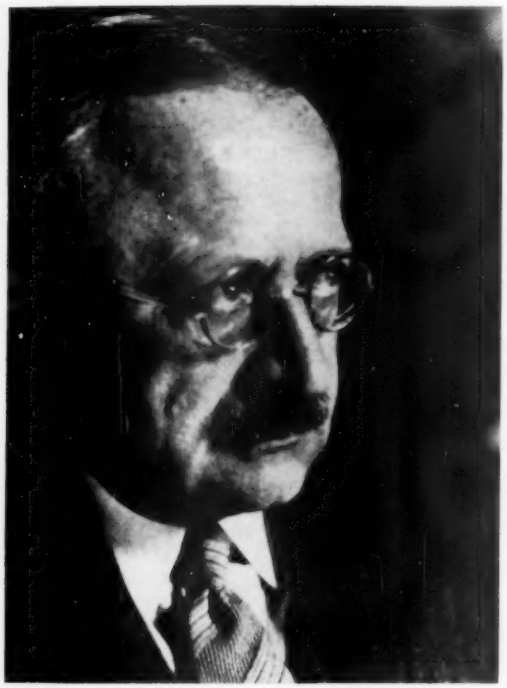
HANS THEODOR GÜSSOW, 1879–1961

A. J. Skolko



Canada lost one of her pioneer botanists in the death of Hans Theodor Güssow at Victoria, British Columbia, on June 15, 1961.

Dr. Güssow was born in 1879 in Breslau, Germany, obtained his early botanical training there and at Leipzig, and later at the Botanical Gardens in Berlin. At the age of 24, he went to England and 2 years later accepted the appointment as assistant to William Carruthers of the British Museum and Consulting Botanist to the Royal Agricultural Society in London. In 1909, he was appointed Botanist in the Dominion Department of Agriculture at Ottawa under William Saunders, Director of Experimental Farms in Canada. He was named Dominion Botanist in 1911 and subsequently Chief of the Division of Botany and Plant Pathology and Associate Director of Science Service in the Canada Department of Agriculture. He served in this capacity until his retirement in 1944.

To document Dr. Güssow's 35-year career is to review the history of botanical, mycological, and phytopathological development and expansion in Canada. Under his organizational and administrative impetus, botanical and phytopathological research grew from a one-man show to a staff of 100 research officers and a chain of 12 research laboratories from the Maritime Provinces to British Columbia at the time of his retirement in 1944. To him, also, must go recognition for the development of the Dominion Arboretum and Botanic Garden and the Mycological and Phanerogamic Herbaria at Ottawa, extensive botanical surveys, seed exchange, potato certification, and for formulation of the Destructive Insect and Pest Act, and Pest Control Products Act. In spite of his heavy administrative commitments, he still found time for his personal scientific interests. Shortly after his appointment in Canada, he discovered on potatoes from Newfoundland the dread wart disease, previously unknown in North America. He then went on to make a study of the disease in Newfoundland. Subsequent studies on common scab of potatoes ("The systematic position of the organism of the common potato scab." Science N.S. 39:431-433. 1914), on cereal diseases ("Smut diseases of cultivated plants, their cause and control." Canada Dept. Agr. Bull. 73. 1913.), and on the fleshy fungi ("Mushrooms and Toadstools of Canada," 1927, with W. S. Odell) attest to the breadth of his interests.

His career was anything but placid; yet, through the most heated encounters he maintained the dignity of his Old Country academic training. Behind the formal, often intimidating, facade, those close to him, especially his subordinates, recognized the warmth and generosity of the man. His honors were many. In 1931, Queen's University bestowed upon him the LL.D. degree. He was a Fellow of the Royal Society of Canada, of the Linnean Society of London and of the Royal Horticultural Society, past president of The American Phytopathological Society, and first president of the Canadian Phytopathological Society.

He is survived by 2 sons and 3 daughters.

EFFECT OF INFECTION BY POTATO VIRUS Y ON THE CONCENTRATION OF POTATO VIRUS X IN TOBACCO PLANTS

Richard F. Stouffer and A. F. Ross

Department of Plant Pathology, Cornell University, Ithaca, New York.

Respectively, former Assistant in Plant Pathology, Cornell University, now Assistant Virologist, Plant Virus Laboratory, Department of Plant Pathology, University of Florida, Gainesville; and Professor of Plant Pathology, Cornell University.

Based on a Ph.D. thesis by the senior author; supported in part by a PHS Research Grant, E-2540, from the National Institutes of Health.

Accepted for publication March 9, 1961.

SUMMARY

Local-lesion assays showed that infection by potato virus Y (PVY) stimulated multiplication of potato virus X (PVX) to only a slight extent in inoculated leaves of Turkish tobacco (*Nicotiana tabacum*), regardless of the sequence of inoculation or whether the first virus was introduced by direct inoculation or *via* systemic movement. Introduction of PVX 7 days before PVY, a sequence that gives maximum interaction in leaves systemically invaded by both viruses during rapid expansion (acute-stage leaves), tended to result in a lesser degree of interaction than did any other sequence.

The ratio of PVX concentration in doubly infected leaves (X_{xy}) to that in singly infected leaves (X_x) was as high as 6.7 during the acute stage (leaves invaded during rapid expansion). For inoculated and for chronic-stage leaves (leaves developing after systemic infection), X_{xy}/X_x ratios were usually less than 2.0. These variations were due largely to variations in X_{xy}. The amount of PVX synthesized as a consequence of PVY infection ($X_{xy}—X_x$) for acute stage, inoculated, and chronic-stage leaves varied, respectively, in the approximate ratio of 100:14:7. In doubly infected plants, PVX concentration was highest in acute-stage leaves; in singly infected plants, highest PVX concentration was in inoculated leaves.

It is hypothesized that infection by PVY markedly stimulates synthesis of PVX only if 3 requisites are fulfilled: 1) introduction of PVY by systemic movement rather than by direct inoculation, 2) initiation of PVY infection during a critical stage in the development of the leaf, and 3) presence of PVX during the rapid phase of PVY multiplication.

It has been known for many years that certain plant viruses in mixed infections interact to induce symptoms more severe than would be anticipated on the basis of the symptoms induced by each virus alone. Bennett (1) first showed quantitatively that infection by one virus can affect the multiplication of another virus. He found that the multiplication of dodder latent mosaic virus is markedly stimulated in mixed infections with certain other viruses. Several other virus pairs are now known in which the multiplication of one virus is affected by the other (2, 3, 4, 12).

Multiplication of potato virus X (PVX) is stimulated to various extents in mixed infections with any of several viruses (3, 4, 5, 6, 7, 10, 12). One of the most striking interactions results from mixed infection of Turkish tobacco (*Nicotiana tabacum* L.) by PVX and potato virus Y (PVY). Previous work at Cornell (6, 7, 10) has shown that PVY infection may cause up to a 10-fold increase in PVX synthesis during the acute stage of the disease, *i.e.*, in uninoculated leaves invaded during rapid expansion. The stimulation was much less marked during other stages of disease. In inoculated leaves and in leaves that expand subsequent to systemic infection (chronic stage), only a 2- to 3-fold increase was recorded. The interaction during the acute stage of the disease was highest in plants inoculated simultaneously with both viruses or with PVX before PVY; the reverse inoculation sequence leads to a mild interaction characteristic of the other 2 stages.

One objective in the present work was to determine the effect of sequence of inoculation on the PVX-PVY interaction in inoculated leaves. Many phases of the interaction could best be studied in such leaves provided consistent, strong interactions could be obtained. Previous studies with inoculated leaves have dealt only with leaves inoculated simultaneously with both viruses.

A second objective was to determine the quantitative aspects of PVX synthesis during the different stages of the disease in both singly and doubly infected plants, with special emphasis on the comparative amounts of PVX formed by a stimulatory effect of PVY infection. Previous data were reported as the ratios of the concentration of PVX in doubly infected leaves (X_{xy}) to that in singly infected ones (X_x). These ratios could be quite misleading if variations in X_x were much greater than those in $X_{xy}—X_x$, the amount of PVX synthesized as a consequence of PVY infection. Hence, it was of interest to determine whether the primary effect of stage of disease is on $X_{xy}—X_x$ or on X_x.

MATERIALS AND METHODS.—The virus strains used, a mild strain of PVX and a fairly typical one of PVY, were maintained in Turkish tobacco and were the same ones used in previous work at Cornell (6, 10). Systemically infected leaves were used for inocula 2-3 weeks following inoculation. The leaves were ground, and the juice was expressed through 2 layers of fine-mesh cheesecloth. The test plant used

in all experiments was Turkish tobacco, grown as previously described (10). Leaves were dusted with 400-mesh Carborundum prior to inoculation with a mixture consisting of 1 part juice from PVX-infected plants plus 1 part juice either from PVY-infected plants or from healthy plants. Plants were rinsed immediately following inoculation.

Test leaves were ground in a food chopper, after which the juice was expressed through cheesecloth, diluted with distilled water, and used for assay within a few hours. For each assay of PVX activity, 12 plants of a specially selected strain of *Gomphrena globosa* L. (11) were trimmed to 4 pairs of opposite leaves and used according to the 2-dilution method of Spencer and Price (9), as adapted for use on *Gomphrena* (7). Each assay compared a test sample with a "standard" or reference inoculum or with another test sample, so that each could be related to a "standard" prepared by a procedure previously found satisfactory (7, 10). At the beginning of each experiment, several tubes of crude juice from PVX-infected tobacco leaves were frozen. On each assay date, 1 of these was thawed, centrifuged 15 minutes at approximately 3,500 *g* in an angle centrifuge, diluted with water, and included in each assay made on that day. Use of a reference inoculum and of the 2-dilution inoculation scheme made it possible to express all data of a given experiment in terms of relative PVX concentration. As a different "standard" was used for each experiment, the data are relative within experiments only, not between experiments. All experiments were carried out in a greenhouse maintained at about 70°F except during summer, when temperatures sometimes exceeded 100°F.

Effect of Sequence of Inoculation on the Interaction In Inoculated Leaves.—*Leaves systemically invaded by one virus and inoculated directly with the other.*—A group of Turkish tobacco plants in the 2- to 3-leaf stages were divided into 5 uniform groups. The 2 lower leaves of plants in 2 of the groups were inoculated with PVX and those of a third with PVY. Seventeen days later, when virus had moved systemically, plants in all groups were decapitated and trimmed to 2 leaves, the fourth and fifth leaves from the bottom. At this time, the remaining 2 leaves on each of the plants in 1 PVX-infected group were inoculated with PVY. Similarly, the PVY-infected leaves and the leaves of 1 group of healthy plants were inoculated with PVX. Another group was inoculated simultaneously with PVX and PVY. On the seventh and eleventh days after the second inoculation, the test leaves were removed from 10 plants in each treatment and assayed for PVX.

Regardless of the sequence of inoculation or whether 1 or both viruses were introduced into leaves by mechanical inoculation, infection by PVY had only a minor stimulatory effect on PVX multiplication (Fig. 1-A). In no case was the X_{xy}/X_x ratio higher than 1.6. The interaction, as measured by the amount of PVX synthesized as a consequence of PVY infection (X_{xy}-X_x), was slightly greater following simultaneous inoculation than when leaves systemically infected by either virus were inoculated with the other. Interaction was least when PVY followed PVX, a sequence that results in maximum stimulation in expanding leaves systemically invaded by both viruses (10). The differences due to inoculation sequence are so small, however, that they may not be significant.

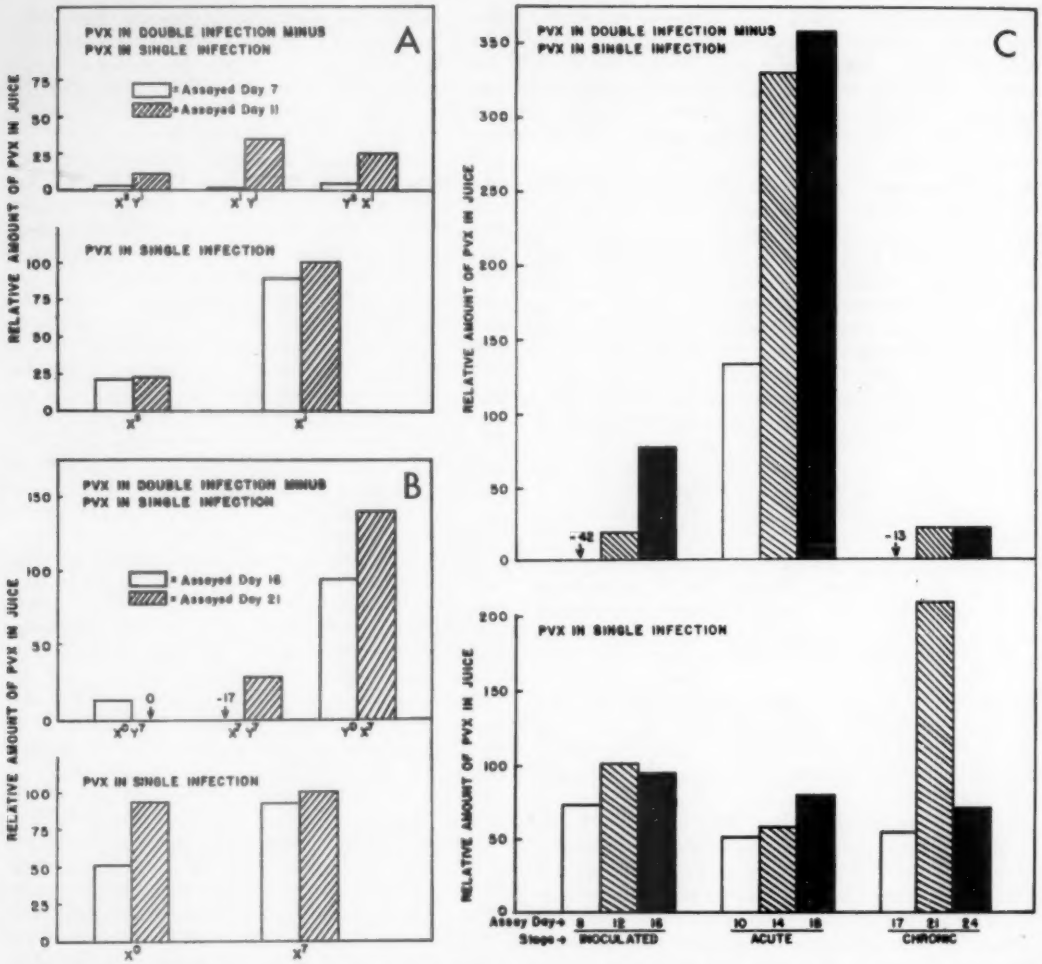
PVX multiplication in singly infected leaves was markedly affected by the manner in which PVX was introduced (Fig. 1-A). When PVX was introduced by direct inoculation, the PVX concentration on each assay date was approximately 5 times greater than that in leaves in which PVX was introduced by systemic movement. Thus, it appears that the mechanism governing the extent of PVX synthesis operates at a lower level in systemically infected leaves than in inoculated ones. Inoculated and systemically invaded leaves were similar with respect to X_{xy}/X_x ratios and $X_{xy}-X_x$ values, but greatly different with respect to X_x values. Apparently, the extent of the PVX-PVY interaction is not related to the level at which the PVX-limiting mechanism normally operates in a given leaf. That is, interaction is not necessarily great under conditions leading to low X_x values.

Results were similar in other tests. PVX concentration was always higher in leaves directly inoculated with PVX than in those invaded systemically by PVX. $X_{xy}-X_x$ values were always small and the differences due to inoculation sequence of doubtful significance. Assays made beyond the eleventh day indicated that PVX concentration sometimes continued to increase up to about the eighteenth day, but there were no further significant changes in the PVX-PVY interaction.

Leaves directly inoculated with the viruses in different sequence.— Turkish tobacco plants in the 6- to 7-leaf stage were selected and divided as in the preceding tests. Each plant was then trimmed to 2 leaves, the fifth and sixth from the bottom. First inoculations were made 2 or 3 days later and the second ones 7 days after the first. The sequence of inoculations was as follows: 1) PVX first, PVY second; 2) PVX first, simulated inoculation by rubbing with healthy-plant juice second; 3) PVY first, PVX second; 4) simulated inoculation first, PVX plus PVY second; and 5) simulated inoculation first, PVX second. The simulated inoculations were made so that rubbing injury would be about the same in all groups. Assays for PVX were made 9 and 14 days later.

As in the previous experiment, PVY infection had no marked stimulatory effect on PVX multiplication (Fig. 1-B). The only appreciable interaction was when PVX followed PVY, a sequence that resulted in X_{xy}/X_x ratios of 2.0 at the first assay and 2.4 at the second. These data are in marked contrast to the earlier ones (6) showing that this sequence results in lower ratios for systemically invaded (acute-stage) leaves than does any other sequence.

That the PVX-PVY interaction in inoculated leaves differs in some fundamental way from that in acute-stage leaves is indicated by 1) consistent failure to obtain high X_{xy}/X_x ratios in inoculated leaves, 2) the failure of sequence of inoculation to affect the inter-


A
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PVX IN SINGLE INFECTION
RELATIVE AMOUNT OF PVX IN JUICE
B
PVX IN DOUBLE INFECTION MINUS
PVX IN SINGLE INFECTION
= Assayed Day 16
= Assayed Day 21
PVX IN SINGLE INFECTION
RELATIVE AMOUNT OF PVX IN JUICE
C
PVX IN DOUBLE INFECTION MINUS
PVX IN SINGLE INFECTION
RELATIVE AMOUNT OF PVX IN JUICE
PVX IN SINGLE INFECTION
RELATIVE AMOUNT OF PVX IN JUICE
Assay Day→ 8 12 16 10 14 18 17 21 24
Stage→ INOCULATED ACUTE CHRONIC

Fig. 1. Relative concentration of potato virus X (PVX) in singly infected tobacco leaves (lower part of each graph) and relative amounts of PVX, in corresponding doubly infected leaves, attributable to the stimulatory effect of infection by potato virus Y (PVY) (upper part of each graph). Letters along base line indicate the viruses used for inoculation (X = PVX, Y = PVY); superscripts indicate how the virus entered the test leaves (i = direct inoculation, s = systemic invasion) or day of inoculation (0 = day 0, 7 = day 7). **A)** In leaves systemically infected by one virus and directly inoculated with the other; X^1Y^1 indicates simultaneous inoculation. **B)** In leaves directly inoculated with X or with both viruses in different sequences. **C)** In leaves sampled according to the stage of the disease. Doubly infected plants were inoculated simultaneously with both viruses.

action markedly, and 3) the tendency of the PVY-before-PVX sequence to have opposite effects in the 2 types of leaves.

PVX Concentration During Different Stages of the Disease in Singly and Doubly Infected Plants. —Turkish tobacco plants in the 3- to 4-leaf stage were divided into 2 uniform groups. Two lower leaves of each plant in 1 group were inoculated with PVX; the other group was similarly inoculated with PVX plus PVY. Beginning 8 days after inoculation and continuing for 16 days, periodic PVX assays were made on leaves in the local, acute, and/or chronic stages of the disease. On each assay date, each group was sampled by careful selection of leaves from 6 plants. Selection of acute- and chronic-stage leaves of doubly infected plants was on the basis of symptoms; location of these leaves served as a guide for sampling of singly infected plants. Usually, acute-stage leaves were the second or third above the uppermost inoculated leaf; chronic-stage leaves, the fifth and sixth.

In general, these results confirm those of Rochow and Ross (6) in that the interaction, as indicated by X_{xy}/X_x ratios, was much more marked in acute-stage leaves than in either of the other 2 types. The X_{xy}/X_x ratios for inoculated leaves and for chronic-stage leaves were generally less than 2.0. In contrast, those

of acute-stage leaves were 3.7, 6.7, and 5.5 on the tenth, fourteenth, and eighteenth days, respectively, after inoculation. Although these are lower than the maximum values reported earlier (6), they are of the same order of magnitude.

PVX-PVY interaction is greatest in acute-stage leaves: PVY infection stimulates synthesis of a much greater amount of excess PVX in acute-stage leaves than it does in either inoculated or chronic-stage leaves (Fig. 1-C). The quantities of PVX formed as a consequence of PVY infection (X_{xy}—X_x) in acute-stage, inoculated, and chronic-stage leaves were in the approximate proportion of 100:14:7.

X_{xy}/X_x ratios, calculated from the last 2 of each set of 3 assays, varied in the same direction as did the values for $X_{xy}-X_x$ but to a slightly smaller extent. Ratios for the respective stages varied in the approximate proportion of 100:26:20. Thus, the earlier conclusion that PVX-PVY interaction is markedly greater in acute-stage leaves than in either inoculated or chronic-stage ones remains valid.

Obviously, variations in the X_{xy}/X_x ratios were due to variations in both X_{xy} and X_x, but the X_{xy} variations accounted for the major part of the variation in the ratio. In doubly infected leaves, PVX concentration (X_{xy}) was much greater in acute-stage leaves than that in the other 2 types. Values for X_{xy} (last 2 assays) for acute-stage, inoculated, and chronic-stage leaves were in the approximate proportion of 100:35:39. In the absence of PVY, however, acute stage leaves contained less PVX than did either of the other 2 kinds. Respective X_x values for acute-stage, inoculated, and chronic-stage leaves were in the approximate proportion of 100:140:200.

Results of several other experiments were similar to those reported, especially for inoculated and acute-stage leaves. Results with chronic-stage leaves varied considerably, as in the experiment reported. This may have been due to sampling error, since leaves of singly infected plants were essentially symptomless. It is only a surmise that the singly infected leaves selected as being in the chronic stage actually developed after systemic infection. It is possible that the chronic stage does not begin in singly infected leaves at the same time as in doubly infected ones. In general, X_x values for chronic-stage leaves were about the same as those for acute-stage leaves or slightly higher. Also, this stage was always characterized by very low X_{xy}/X_x ratios and $X_{xy}-X_x$ values.

Discussion.—Previous results (6) indicated that PVY infection has a marked stimulatory effect on PVX synthesis only during the time when PVY is multiplying rapidly. Thus, for strong interaction, PVX must be present in a host cell while that cell is supporting rapid PVY synthesis. The present results in no way contradict this conclusion, but they do show that this is not the only requirement. Some other requisite was missing in the leaves inoculated directly, at least in those directly inoculated with PVY. In leaves simultaneously inoculated with both viruses, the requirement stipulated above may not be met in all cells of the leaf inoculated; undoubtedly, some cells would be invaded by PVX only after completion of the rapid phase of PVY multiplication. Probably, however, most cells would meet the requirement and at least a moderate interaction should result. More importantly, the sequence of PVX before PVY, especially when PVX was introduced *via* systemic invasion, should have established PVX in all cells prior to their invasion by PVY. The failure of this sequence to result in a strong interaction points to some other fundamental difference between inoculated and acute-stage leaves.

Leaves directly inoculated and those selected as acute-stage leaves differed in size or age at the time when double infection was established. The leaves on trimmed and decapitated plants were usually about 8 cm wide when inoculated; expansion had not ceased, however, for the leaves approximately doubled in size during the experiments. Also, inoculated leaves on intact plants did not expand appreciably; yet, the interaction in them was similar to that in leaves on trimmed plants. Hence, leaf expansion *per se* does not appear to be the missing requirement. The leaves may have been too old, as suggested by indirect evidence indicating existence of a critical stage in the leaf's development during which PVY must be multiplying rapidly for a strong interaction to result. An early limit to the supposed critical stage is indicated by the lack of a strong interaction in leaves invaded very early, *i.e.*, chronic-stage leaves. Furthermore, symptoms characteristic of the acute-stage of disease in doubly infected plants do not develop in leaves that are mature or nearly so when systemically invaded by the 2 viruses; PVX-PVY interactions characterized by marked increases in symptom severity, in general, have been indicative of a marked increase in PVX synthesis. Leaf age may be a factor affecting the interaction, as further indicated by the results of experiments by Rochow (4) and by us in which leaves 1-8 cm in diameter were inoculated. The highest X_{xy}/X_x ratios were obtained from the smallest leaves; however, these ratios were not comparable with those obtained with acute-stage leaves.

Possibly, a strong PVX-PVY interaction may obtain only if PVY enters the leaf *via* the vascular system. This possibility is suggested by the earlier observation (6) that the stimulatory effect of PVY infection on PVX multiplication is stronger in veinal and adjacent tissue than in interveinal tissues. Also, symptoms of double infection not only appear first in veinal and adjacent tissues but also are most severe in such tissues; in inoculated leaves and in chronic-stage leaves, symptoms are predominantly interveinal, as are those few symptoms induced by PVX alone. Following systemic invasion, infection is probably established in or near the veins whereas mechanical inoculation initiates infection mostly in interveinal tissue. In other virus-host systems, virus infection in one part of a leaf altered the rest of the leaf and affected multiplication of a subsequently introduced virus (8). Perhaps, the ability of PVY to stimulate PVX synthesis in one kind of tissue, *e.g.* veinal tissue, depends on whether PVY has multiplied previously in

nearby tissues in the same leaf.

The finding that the main influence of temperature on the X_{xy}/X_x ratio is an effect on X_x (10) suggested that a similar situation may exist with respect to stage of disease. Our results show that this is not the case, for X_{xy} values varied more than X_x values. If different stages of the disease had been characterized by different X_x values and similar X_{xy} values, the data would have supported the hypothesis (6, 10) that PVY infection acts to nullify the mechanism that normally limits PVX synthesis, allowing PVX synthesis to proceed until another limiting mechanism becomes operative. Thus, the existence of a second limiting mechanism, similar in all leaves, would have been indicated. The data obtained do not deny the hypothesis, however; possibly, some factors influence the first PVX-limiting mechanism and others affect the second, or perhaps the mechanism that eventually limits PVX synthesis (after stimulation by PVY infection) differs from leaf to leaf or from condition to condition. For example, our data indicate that the route of introduction of PVX affects the level at which the first limiting mechanism normally operates, *i.e.*, systemic invasion leads to lower PVX levels in singly infected leaves than direct inoculation does. Perhaps the identity and/or level of operation of the second PVX-limiting mechanism is determined by the stage of leaf development during which the double infection is established.

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VERTICILLIUM WILT OF STRAWBERRY CONTROLLED BY FUMIGATION OF SOIL WITH CHLOROPICRIN AND CHLOROPICRIN-METHYL BROMIDE MIXTURES

Stephen Wilhelm, R. C. Storkan, and J. E. Sagen

Respectively, Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley; Director of Technical Services and Development, Neil A. Maclean Company, Belmont, California, now President, Tri-Cal, Inc., Los Gatos, California; and Laboratory Technician, Department of Plant Pathology, University of California, Berkeley.

SUMMARY

In laboratory and field tests the fungus, *Verticillium albo-atrum*, has shown fine susceptibility to chloropicrin. Chloropicrin is used commercially to control *Verticillium* wilt of strawberry in California. For effective soil fumigation with chloropicrin, soil must be finely tilled and moist to the surface. Covering fumigated soil with polyethylene sheeting and adding methyl bromide to chloropicrin augment *Verticillium* wilt control, but do not obviate the requirement that soil be moist. Strawberry plant vigor and longevity in fumigated soils have been outstanding and yields have exceeded commercial production by nearly 4 times.

Chloropicrin (trichloronitromethane) has been used commercially for the control of *Verticillium* wilt of strawberry caused by the microsclerotial *Verticillium albo-atrum* Reinke & Berth. since 1953 when one acre was first successfully fumigated by hand guns (8). After 1953, the hand method of fumigation which consisted of point-injecting chloropicrin at 12-in. staggered spacings was compared with machine application with chisels spaced 12 in. on the tractor tool bar. By machine, the injection rate of chloropicrin was constant and the flow continuous. Under both hand and machine methods of application, *Verticillium* wilt control was equivalent when 480 lb of chloropicrin/acre were applied. To cite 1 instance, 0.5 acre of strawberries planted on *Verticillium* infested land, fumigated by hand guns in October 1955, resulted in 3.9% of diseased plants during the first year (mean 17.2±8.9/bed of 440 plants). In 2 adjacent acres fumigated by tractor-mounted equipment, there were 1.6% diseased plants (mean 7.0±3.7/bed of 440 plants), and in a nonfumigated check area there were 14.1% (mean 62.1±10.9) diseased plants. The vigor and productiveness of the fumigated areas were outstanding, and they were maintained in commercial production for 5 years. Plants in nonfumigated areas lacked vigor and were maintained only 2 years.

VERTICILLIUM IN DRY SURFACE SOIL MAY SURVIVE HEAVY APPLICATIONS OF CHLOROPICRIN.— Laboratory tests were designed to compare the effectiveness of chloropicrin against *Verticillium* in moist and dry soil. The technique used was essentially that reported earlier (6), except that open bottom cylinders made of 0.25-in. mesh heavy galvanized wire were used instead of large cans to contain the test soil. The wire cylinders were 14 in. in diameter and 16 in. high. Prior to use, they were lined with waxed paper and placed upon soil contained in flats. Thus, there was no artificial impediment to the downward escape of the fumigant.

Test inoculum was prepared by growing an isolate of *V. albo-atrum* virulent to tomato on autoclaved pieces of previously dried, chopped tomato stems. Microsclerotia formed abundantly within and on the tissues. The inoculum was dried at room temperature for 2 days before being used in the tests. The test soil was a uniform medium heavy loam. In test I (Fig. 1), the moisture content of the soil was about 20% of the dry weight (approximately 50% field capacity) and was indicated as moist. In test II, the upper 4.5 in. of soil contained only approximately 4% water and was indicated as dry, but below this level the soil was moist. In test III, the soil throughout the cylinder was dry. As the cylinders were filled with soil, 3 inoculum pieces each were placed at 12, 9, 6, 3, and 1.5 in., respectively, from the soil surface; and spaced equidistant from each other at the periphery of a circle 1 ft² in area. The inoculum units were allowed 1 day to come to equilibrium with the moisture of the soil. The test was conducted outdoors. Chloropicrin was injected in the center of the filled cylinders 6 in. deep at dosages of 0, 2, 3, and 4 ml. This was equivalent to 0, 320, 480, and 640 lb/acre, respectively. After injection, the soil was firmed but not covered. The exposure period was 10 days. Soil temperatures at the 6-in. depth ranged from a low at night of 16°C to a high of 24°C during the day. The experiment was replicated twice.

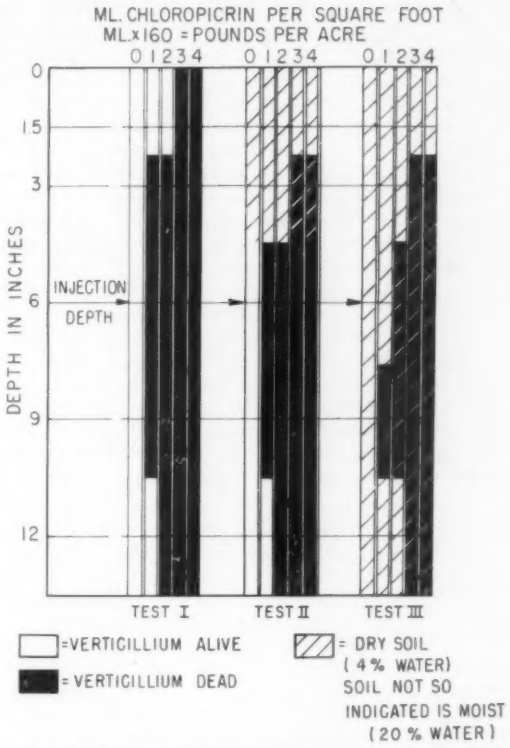

Fig. 1. Diffusion pattern of chloropicrin and concentrations required to kill *Verticillium* in moist and dry soils.

After the period of exposure to chloropicrin, the *Verticillium* test inoculum was recovered from the soil and tested for viability in 3 different ways:

1) Each inoculum piece was washed in 20 ml of sterile water, dried on a paper towel, and cut lengthwise. One of the halves was plated on plain agar containing bits of sterilized barley straw. *Verticillium* was considered viable if it colonized the barley straw fragments and produced its characteristic microsclerotia.

2) The half-piece not cultured was inserted into a longitudinal cut made near the base of the stem of a young tomato plant, and secured by binding Scotch tape around the stem. *Verticillium* was considered viable if the plant developed symptoms of *Verticillium* wilt, and yielded the fungus upon culture.

3) Roots of 5 young tomato seedlings were dipped into the water used to wash the inoculum pieces and planted around the periphery of a 6-in. pot of sterilized soil. The remaining wash water was poured over the soil in the pot. *Verticillium* was considered viable if any of the 5 tomato plants of a pot showed symptoms

of *Verticillium* wilt and yielded the fungus in culture.

In presenting the results (Fig. 1), *Verticillium* was considered killed by the chloropicrin only if the 3 inoculum units at a given depth in both replications lacked viability, as determined by at least 2 of the 3 methods outlined above. Conversely, *Verticillium* was considered alive if viability could be detected in any 1 of the 3 inoculum pieces by any 1 of the 3 methods. Consequently, the data presented are conservative and represent the lowest level of performance of chloropicrin in both replications. Agreement between replications was very good.

The results (Fig. 1) showed that 3 ml of chloropicrin/ft^2 or 480 lb/acre injected 6 in. deep is the minimum dosage required to kill *Verticillium* in moist soil to a depth of 12 in. At 1 and 2 ml/ft^2, 160 and 320 lb/acre, respectively, the *Verticillium* in the surface soil and below 9 in. may escape. In dry soil, the performance of chloropicrin was poorer than in moist soil; near the surface, *Verticillium* escaped even at dosages of 3 and 4 ml/ft^2, or 480 and 640 lb/acre, respectively.

Many Fungi Tolerate Chloropicrin.—Since these tests were conducted in natural soil, culturing of the *Verticillium* inoculum pieces after exposure to chloropicrin may indicate other soil fungi not killed by the chloropicrin. At the 3 ml/ft^2 dosage, which was consistently lethal to *Verticillium* at soil depths of 1.5-12.0 in., species of the fungus genera *Trichoderma*, *Gliocladium*, *Melanospora*, *Stysanus*, *Chaetomium*, *Ceratostoma*, *Graphium*, and certain actinomycetes consistently survived. These fungi no doubt figure strongly in the biological equilibrium ultimately established in chloropicrin-fumigated soil. Certain fungi, particularly *Gliocladium* and *Chaetomium*, show a measure of antagonism to *Verticillium* (7).

Chloropicrin-Methyl Bromide Mixtures Control Verticillium Wilt.—Methyl bromide—dissolved in certain hydrocarbon diluents in the proportions of approximately 70% methyl bromide and 30% diluent by weight, applied by tractor-mounted equipment to the soil, and covered with polyethylene sheeting—has been used successfully for the control of broomrapes in California (9, 10). Known commercially as Weedfume, this fumigant, at dosages of 175-225 lb/acre of actual methyl bromide, also has shown great effectiveness against unsprouted seeds of many different kinds of weeds, but is completely without effect on *Verticillium*. Our field observations indicate that its use may effect an increase in both amount and severity of the disease.

In 1957, chloropicrin alone and the mixture of chloropicrin, 2 parts, and methyl bromide, 1 part, by weight were applied in the field by tractor-mounted equipment. Outstanding *Verticillium* wilt and weed control was obtained by the mixture in experimental plots planted to strawberries in soil heavily infested with *Verticillium*. One section of the experimental area was irrigated prior to application of the fumigants and contained 12-15% of moisture a few inches below the soil surface, or a little under 50% of the estimated field capacity of the soil. An adjacent but smaller section was dry and cloddy. Differences in moisture content and tilth of the soil also permitted comparison of the performances of chloropicrin and the chloropicrin-methyl bromide mixture in moist and dry soils, but did not allow for replications of each treatment. Treatments were designed to obtain data on the following: 1) the control of *Verticillium* wilt by the 480 lb/acre rate of chloropicrin applied to dry as compared with moist soils, 2) the effect of covering fumigated soil with polyethylene sheeting, and 3) the effect of applying a mixture of chloropicrin and methyl bromide. The plots were 50 ft long, 10 ft wide, and planted to 300 Shasta strawberry plants in 3 double-rowed beds. In the moist area of the field, 480 lb/acre of chloropicrin, our commercially recommended rate applied without covering, was compared with 480 lb covered and with 480 lb/acre of the chloropicrin-methyl bromide mixture covered. This mixture contained 320 lb of chloropicrin and 160 lb of methyl bromide. Similarly, application of 320 lb of chloropicrin alone/acre covered and not covered was compared with 320 lb/acre of the above chloropicrin-methyl bromide mixture covered. These treatments except for the 1 indicated (Table 1) were replicated twice. In the dry cloddy area of the field, chloropicrin applied at the 480 lb/acre rate uncovered was compared with 480 lb of the chloropicrin-methyl bromide mixture which was covered. The dry-area treatments were not replicated. Four nonfumigated plots served as checks. To aid in the collection of data suitable for statistical analysis and because of possible variations in inoculum levels of *Verticillium* within plots or of fluctuations in delivery of the fumigant during application, each plot was divided into 6 equal blocks, each containing 50 plants. The plots were maintained for 2 years. Each plant showing *Verticillium* wilt was counted and its position in the plot recorded. In many instances, the field diagnosis of *Verticillium* wilt was confirmed by laboratory isolation of *V. albo-atrum* in pure culture from petioles.

Results.—The nonfumigated check plots succumbed quickly to *Verticillium* wilt. By the end of the first growing year, an average of only 26.8% of plants remained alive. Plants continued to die into the second year. Three hundred and twenty lb/acre of chloropicrin uncovered (treatment B, Table 1) gave relatively poor control of *Verticillium* wilt (13.4% of diseased plants), but the same treatment covered (treatment C) gave significantly better control (7.6% of diseased plants). This compared favorably with results obtained by the use of 480 lb/acre uncovered (treatment G). Covering also improved the control obtained with 480 lb/acre (treatment E). Applied to dry, cloddy soil, 480 lb/acre of chloropicrin failed to control *Verticillium* wilt (treatment I).

Methyl bromide, which is not lethal to *Verticillium* at rates up to 225 lb/acre, applied as commercial Weedfume (70% methyl bromide in hydrocarbon solvents) in combination with chloropicrin, formed a mixture apparently more lethal than chloropicrin alone. Application of 320 lb/acre of the mixture (treatment D)

TABLE 1. The control of *Verticillium* wilt of strawberry, plant growth response, and yield following soil fumigation in 1957 with chloropicrin and a mixture of chloropicrin, 2 parts, and methyl bromide, 1 part. Means of the same array are significantly different from other means of the same array if not followed by the same alphabetical letter

Treatment (A-F inclusive was in moist soil, G-J in dry)	No. of plants with *Verticillium* wilt (No. × 2 = percentage)				Estimation of plant vigor (measurements in cm)								Yield[d] (lb/acre) Avg. of 2 replicates
					Leaf diameters in cm				Plant height in cm				
			Signif.				Signif.				Signif.		
	Rank	Mean	.01	.05	Rank	Mean	.01	.05	Rank	Mean	.01	.05	
A. Check-nonfumigated	1	30.1	a	a	1	13.3	a	a	1	20.9	a	a	.....
B. 320 lb/A PIC[a]	2	6.7	b	b	2	15.3	b	b	2	22.7	b	b	33,700
C. 320 lb/A PIC-T[b]	3	3.8	c	c	3	15.7	bc	bc	3	22.9	bc	b	38,900
D. 320 lb/A PIC-MB[c]-T	4	3.0	d	d	4	15.7	bc	bc	5	23.3	bc	bc	43,700
E. 480 lb/A PIC-T	5	2.8	d	e	5	15.9	bc	c	4	23.2	bc	bc	36,300
F. 480 lb/A PIC-MB-T	6	1.0	e	f	6	16.3	c	c	6	23.7	c	c	46,300
G. 480 lb/A PIC[e]		5.2				15.9				23.1			32,700
H. Check-nonfumigated[e]		38.2											
I. 480 lb/A PIC[e]		17.8											
J. 480 lb/A PIC-MB-T[e]		6.5											

[a] PIC = Chloropicrin.
[b] T = Tarped (covered).
[c] PIC-MB = 2 parts chloropicrin, 1 part methyl bromide.
[d] Average commercial yield in the area was under 12,000 lb/A.
[e] Not included in statistical analysis because plots were not replicated. The values are the mean of 6 blocks within 1 treatment.

which contained 214 lb of chloropicrin and 106 lb of methyl bromide, neither of which applied singly adequately controls *Verticillium*, gave control equal to that obtained with 480 lb of chloropicrin covered (treatment E). Only 2% of diseased plants resulted from 480 lb/acre of the above mixture covered (treatment F), but in dry soil it was much less effective (treatment J). According to the analysis of variance, our conclusions were sound.

Weed control.—With the chloropicrin-methyl bromide mixture (treatments D and F), nearly complete control of the following weeds also was achieved: *Amaranthus blitoides* Wats., *A. retroflexus* L., *Chenopodium murale* L., *Capsella bursa-pastoris* L., *Matricaria* sp., *Oxalis corniculata* L., *Portulaca oleracea* L., *Senecio vulgaris* L., *Solanum sarachoides* Sendt., *Stellaria media* L., and volunteer tomatoes. Because of the outstanding herbicidal and fungicidal properties of chloropicrin-methyl bromide mixtures, they were used on most of the strawberry lands in California fumigated in 1959-60.

Plant vigor and yield following fumigation.—Plant vigor was estimated by measuring the greatest distance across 3 strawberry leaves and the heights of each of 50 consecutive plants of each plot. Measurements were made 3 months after planting, before any visible effects of *Verticillium* were apparent in the check plots. The significantly greater growth of strawberry plants in all plots receiving fumigation, represented by larger leaves and taller plants (Table 1), may reflect the control, not only of *Verticillium*, but of other pathogens resident in the soil. This interpretation of the growth response has been given by Howard and Crandall (1) and others (3) to explain fumigation responses in other crops. Yields were estimated by weighing the total strawberry production, in the second year up to September 1, of plants in 10 ft of uninterrupted bed of each plot. Yield estimates (Table 1) were based on a production of 25,000 strawberry plants/acre and were not corrected for losses due to *Verticillium* wilt. The figures also have been rounded off to the nearest 100 lb. There were no yields from the checks, which in the second year contained fewer than 10% viable plants. Yields in the fumigated plots exceeded the average county yield by 3 times, and in 2 instances nearly 4 times.

DISCUSSION.—The control of a plant disease by chemicals always is of great interest. 1) It makes possible the cultivation of susceptible varieties of a crop which often are the commercially desirable ones. 2) It might release the efforts of applied crop breeders to concentrate on factors of fruit quality and yield. 3) It usually is effective against all races and forms of the pathogen. 4) It stimulates growers to understand problems in plant disease control and to adapt farm procedures consistent with the scientific solution of the problems. 5) When soil-borne plant pathogens are involved, it makes available for cultivation lands that otherwise would be avoided because of disease. 6) It may give growth responses and yields far beyond those anticipated.

The serendipic effects of soil fumigation have been particularly notable in strawberry growth and yield both in these tests and wherever chloropicrin has been used commercially, and these effects are thought to reflect both the control of perhaps numerous kinds of soil-borne pathogens and the salutary effects among the surviving nonpathogens.

Laboratory results from tests, as outlined, gave a reliable indication of the performance of chloropicrin in the field and indicated both field dosage and necessary land preparation. In dry surface soil, *Verticillium*

may escape the lethal vapors of chloropicrin, since organisms in a dry state and inactive are more resistant to the injurious effects of heat and chemicals than moist organisms. In tests in air, Stark (4) showed that high humidity increased the toxicity of chloropicrin. In laboratory tests the 320 lb/acre rate of chloropicrin may only fail to control *Verticillium* in the surface soil. In the field, 320 lb/acre, when covered with polyethylene sheeting, gives excellent control of *Verticillium*.

Verticillium is particularly resistant to methyl bromide (2) and rates/acre up to 200 lb, in our observations, have appeared to increase greatly the incidence and severity of the wilt disease. Applied with chloropicrin, a fumigant is formed which is more effective against *Verticillium* than chloropicrin alone. Whether this increased activity is due to a synergism between the chemicals, or to formation in the mixture of new chemicals such as bromopicrin, chlorobromopicrin, or to free bromine or chlorine is not known. Significant weed control also was achieved by this mixture. Stark, *et al.* (5), among the first to mix chloropicrin and methyl bromide, suggested that the mixture was more nematocidal than either component alone.

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THE RELATIVE SURVIVAL ABILITY OF RACES OF PHYTOPHTHORA INFESTANS IN MIXTURES

H. David Thurston

Former Research Fellow, Department of Plant Pathology and Botany, University of Minnesota, St. Paul; now, Plant Pathologist with Rockefeller Foundation, Bogotá, Colombia, South America.

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SUMMARY

In 7 experiments made in the greenhouse with mixtures of races 0 and 1; 0 and 4; 0 and 1,4; 0 and 2,4; and 0 and 1,2,4, race 0 predominated or entirely displaced the other races with which it was mixed after perpetuation of the mixture on susceptible potato clones for 2-9 generations. When compared singly, isolates of race 0 were usually more infectious than the other races and there were indications that race 0 was antagonistic to other races with which it was mixed. Water in which sporangia of individual races had germinated reduced germination of fresh sporangia of the same races and of most other races.

When a field plot of the Cobbler (r) variety was inoculated near the center with races 1 and 4, race 0 appeared from an unknown source and predominated, indicating superior aggressiveness.

In another field test, race 2,4 spread most rapidly, with race 1 ranking second. Races 4 and 1,2,4 spread more slowly and race 0 slowest of all. This indicates that certain isolates of race 0 may be relatively nonaggressive even though they usually predominate over other races.

Phytophthora infestans (Mont.) de Bary, the fungus that causes late blight of potatoes, comprises numerous physiologic races distinguished by their pathogenicity on potato clones with different dominant genes for resistance inherited from *Solanum demissum* Lindl. Black, *et al.* (4) proposed an international nomenclature for these races, many of them occurring naturally in Mexico (19), the United States (29), and Canada (14).

In the United States and other countries, when potato varieties deriving their resistance from *S. demissum* have been introduced into commercial production, races capable of attacking them have appeared. If, in addition to their wider host range, the new races

survive better and are also more aggressive than those previously prevalent, continued breeding and introduction of such varieties could lead to a population of races of potentially greater menace to the potato crop. If, on the other hand, they are less fitted to survive and spread than the races on the older varieties, the new varieties should be damaged less by blight, even though susceptible to the new races. It is therefore important to obtain basic information on the probable capacity of these races to survive in nature and cause epidemics.

"Relative survival ability" of a race is used here to designate the ability to survive and cause disease under conditions fatal to, or in competition with, another race. Survival ability is the result of a complex of factors, any one or combination of which may be of importance to the survival of a given race, or isolate of that race. These factors may be influenced by the various interactions of host, parasite, and environment; and probably include ability of a fungus to overseason (live during seasons or periods unfavorable for its growth); to grow after overseasoning; to sporulate; to survive dissemination until arrival on a susceptible host; to germinate rapidly; and finally to penetrate and infect the host.

In this study, it was necessary to differentiate between the degree of pathogenicity of a parasite once established inside the host, and pathogenicity as indicated by the prevalence of the pathogen on a large host population. The term aggressiveness is used in this paper as defined by Knutson (16): "the relative ability of the pathogen to increase to epidemic proportions in a host population." Gäumann (12) used aggressiveness to mean "the capacity (of a pathogen) to invade, *i.e.* to infect its host, to inhabit or live in it, to overcome its resistance, to make use of it for its own nourishment, and to reproduce itself in or on it." The Winston dictionary's definition of aggressiveness implies more than this: "quickness to attack, moving forward with vigor, ready to take the initiative." The other term closest to this meaning in plant pathology—virulence—has been defined by Ainsworth and Bisby (1) as "the degree or measure of pathogenicity" and by the Committee on Technical Words of The American Phytopathological Society (21) as "the relative capacity to cause disease; degree or measure of pathogenicity of a parasitic organism or virus." These meanings of virulence do not differentiate between the degree of pathogenicity within a single host and on a host population. For these reasons, Knutson's definition is used herein.

Knutson (16) recently reviewed the literature on factors influencing aggressiveness of different races. Although many of the same factors that affect survival ability affect aggressiveness, it does not necessarily follow that a race with high survival ability is the most aggressive. Nevertheless, survival ability is probably one of the most important factors in the over-all aggressiveness of races.

Several investigators have found differences in the survival ability of different races of various species of fungi when mixtures were perpetuated on susceptible hosts (3, 5, 6, 15, 17, 22, 28). Similarities between the behavior of races in mixtures and behavior in nature, however, were difficult to demonstrate.

Thurston and Eide (25) reported that a mixture of races 0 and 1 of *P. infestans*, transferred for 4 successive generations on a variety susceptible to both races, produced 20 times as many lesions on Cobbler (r) as on Cherokee (R_1), as a result of the final inoculation with the mixture. They (26) reported a similar experiment with the same races in which, after 8 successive generations, the proportion of race 0 to race 1 in the mixture was 90:1.

Black (3) worked with a mixture of 2 races for 4 generations and a mixture of 6 races for 10 generations. He found that the wider the host range of a race, *e.g.*, race 1,3,4, the less prolific it was on varieties susceptible to more races, and the lower its survival value in competition with races with a narrower host range.

The present study was made to obtain basic information on the relative survival ability and aggressiveness of the races of *P. infestans* present in Minnesota.

MATERIALS AND METHODS.—All cultures of *P. infestans* studied were isolated in Minnesota and maintained on lima-bean agar (24). They were identified as races of *P. infestans* by their pathogenicity on differential potato clones (4). Races pathogenic on varieties with major (R) resistance genes are referred to as "specialized races" in this paper, following the custom of other authors (11).

The system used to designate presence of major genes for resistance in the potato clones used in this study, *i.e.*, Cherokee (R_1), is that of Black, *et al.* (4). The designations r and (r) indicate lack of major genes for resistance.

Inoculum was produced either by maintaining and increasing the fungus on plants in the greenhouse or by growing it on autoclaved yellow peas (24). Sporangia produced on leaves or yellow peas were suspended in water, and concentrations of the 2 races to be compared were equalized by counting 10 samples with a haemocytometer and appropriately diluting. Equal volumes of the 2 suspensions were mixed to make a suspension having, presumably, an equal number of sporangia of each race. Also, plants were inoculated with suspensions of each race alone. In the earlier experiments, the suspensions of each race alone were first diluted with an equal amount of water, so each contained the same concentration of sporangia of that race as did the mixture. Later, the original suspensions were used undiluted.

Six plants of each of 2 clones were inoculated with each suspension. With 1 exception (experiment 10, with 5 leaves only), each plant had at least 6 well-developed leaves with terminals and 2 lateral leaflets intact.

Plants were first sprayed with tap water; then, the lower sides of the leaves were sprayed uniformly with the suspension with a Flit-gun type of sprayer. In experiment 2, a deVilbiss atomizer with 7 lb air

pressure from a motor-driven pump was used to spray each plant for 10 seconds. Care was taken to avoid contaminating plants with sporangia of the other races. Inoculated plants were immediately placed in separate metal moist-chambers overnight. Except where noted, plants were grown and inoculated in a greenhouse at 18-21°C. From the moist chambers, the plants were transferred to greenhouse benches at the same temperature.

After 3-4 days, or whenever the lesions became visible, the lesions on the terminal and 2 lateral leaflets of 6 leaves were counted. The lesions were usually separate and easy to count, but occasionally coalesced, making it difficult to do more than estimate the number of individual lesions.

After lesion counting, sporangia were collected from lesions on the clone susceptible to both races and inoculated with the mixture. These sporangia came either from leaves of infected plants placed in metal moist-chambers in the greenhouse, or from infected leaves detached at random and placed in petri-plate moist chambers at 20°C. Suspensions of the sporangia were used to inoculate additional plants susceptible to both races. As this procedure was followed, successive generations of fungus were carried on until a final test in which the relative proportions of the races could be determined by inoculating clones of the appropriate genotype.

RESULTS.—*Survival of races in mixtures.*—Table 1 summarizes the results of the 11 experiments made on the relative survival ability of races in a mixture. In experiment 2 (Table 1), the relative proportions of each race were tested at each transfer generation. This test indicated that the relative numbers of lesions produced by the mixture on Cobbler (r) and Cherokee (R_1) fluctuated from generation to generation of the fungus instead of changing uniformly. In the same experiment, no appreciable change in the final number of lesions occurred when race 1 was perpetuated separately on Cobbler and on Cherokee for 5 generations.

The following facts are apparent from the data in Table 1: 1) in all tests, the 5 different isolates of race 0 predominated or were the only survivors after 2-9 generations; 2) experiments 3 and 4 (made simultaneously with the same inoculum and methods) indicate that infection may be reduced at a higher temperature, but that the final inoculation differed little at 18° and 23°C.

It is difficult to explain the predominance of race 0 in the mixtures; however, at least 2 possibilities are apparent: 1) the cultures of race 0 predominated in mixtures with other races because their spores germinated better and faster and penetration of the host tissue was quicker; 2) race 0 had a deleterious effect on the other races.

A comparison of the initial infections produced by the individual races and mixtures (Table 2) provides evidence for both of these possibilities:

1) Isolates of race 0 usually caused more infection on the r varieties (Cobbler, *etc.*) than did the other races tested (tests 2, 3, 4, 5, 6, 8, 9, and 10). These differences were statistically significant in tests 3, 5, 6, 9, and 10. In 3 tests (1, 7, and 11), infection by the specialized race on the r host exceeded that by the 0 isolate. These results suggest that the 0 isolates tested were generally more aggressive than the specialized races, but not invariably so.

2) When plants were inoculated with a mixture of 2 races, infection was usually less than the calculated amount based on the infection by each race separately (Table 2). This was evident on both the Cobbler (r) or other r variety, and on the varieties with major (R) genes for immunity.

TABLE 1. Infections on differential potato clones by mixtures of 2 races of *Phytophthora infestans* before and after serial transfers on a variety of potato susceptible to both races

Expt. no.	Races	Lesions/plant from initial inoculation[a]		Intermediate generations on R_0 host	Lesions/plant from final inoculation[a]	
		Cobbler (R_0)	Cherokee (R_1)		Cobbler (R_0)	Cherokee (R_1)
1	0 & 1	272	111	3	85	5.0
2	0 & 1	147	49	7	330	3.7
3[b]	0 & 1	272	51	1	838	3.5
4	0 & 1	37	17	1	85	0.2
5	0 & 1	197	12	8	210	0
6	0 & 1	43	23	8	282	0
		Ostbote (R_0)	Essex (R_1)		Cobbler (R_0)	Cherokee (R_1)
7	0 & 1	46	43	8	41	0
		Cobbler (R_0)	10–5–12 (R_4)		Cobbler (R_0)	10–5–12 (R_4)
8	0 & 4	164	24	4	594	1.3[c]
		Cobbler (R_0)	3WM–19 ($R_{1,4}$)		Cobbler (R_0)	3WM–19 ($R_{1,4}$)
9	0 & 1,4	118	70	1	238	0
		Cobbler (R_0)	3XX–4 ($R_{2,4}$)		Cobbler (R_0)	3XX–4 ($R_{2,4}$)
10	0 & 2,4	32	10	2	161	0
		Cobbler (R_0)	3XX–1 ($R_{1,2,4}$)		Cobbler (R_0)	3XX–1 ($R_{1,2,4}$)
11	0 & 1,2,4	44	61	8	274	0

[a]Mean lesions/plant for 6 plants. Lesions/plant refers to the total number of lesions on the terminal and first 2 lateral leaflets of 6 leaves on a plant.

[b]This experiment was made at 23°C; all others were made at 18–21°C.

[c]A small amount of race 4 is constantly associated with race 0, as noted by Thurston (23).

TABLE 2. Actual and theoretical initial infections on differential clones of potatoes by races of *Phytophthora infestans*, singly and in mixtures

Test	Race and isolate	Sporangia /ml in inoculum	Clone and genotype of host	Lesions/plant [a]						
				Actual	Significance [b]			Theoretical [c]	Act. / Theo. × 100	
1	0 (458)	90,000	Cobbler r	214	a	b	c			
	0		Cherokee R_1	0						
	1 (460)		Cobbler r	472	**					
	1		Cherokee R_1	321		b	d			
	0 + 1	180,000	Cobbler r	272			c	686	40	
	0 + 1		Cherokee R_1	111			**	321	35	
2	0 (458)	14,000	Cobbler r	160	a	b	c			
	0		Cherokee R_1	0						
	1 (460)		Cobbler r	106	a					
	1		Cherokee R_1	93		b	d			
	0 + 1	28,000	Cobbler r	147			c	266	55	
	0 + 1		Cherokee R_1	49			d	93	53	
3	0 (479)	10,500	Cobbler r	369	a	b	c			
	0		Cherokee R_1	0						
	1 (475)		Cobbler r	180	*					
	1		Cherokee R_1	222		b	d			
	0 + 1	21,000	Cobbler r	272			c	549	50	
	0 + 1		Cherokee R_1	51			**	222	23	
4	0 (479)	10,500	Cobbler r	64	a	b	c			
	0		Cherokee R_1	0						
	1 (475)		Cobbler r	36	a					
	1	10,500	Cherokee R_1	66		b	d			
	0 + 1	21,000	Cobbler r	37			c	100	37	
	0 + 1		Cherokee R_1	17			**	66	26	
5	0 (481)	5,000	Cobbler r	311	a	b	c			
	0		Cherokee R_1	0						
	1 (469)		Cobbler r	54	**					
	1		Cherokee R_1	30		**	d			
	0 + 1	10,000	Cobbler r	197			*	365	54	
	0 + 1		Cherokee R_1	12			**	30	40	
6	0 (481)	22,000	Cobbler r	45	a	b	c			
	0		Cherokee R_1	0						
	1 (469)		Cobbler r	28	*					
	1		Cherokee R_1	77		**	d			
	0 + 1		Cobbler r	43			c	37	116	
	0 + 1		Cherokee R_1	23			**	38	60	
7	0 (481)	22,000	Ostbote r	24	a	b	c			
	0		Essex R_1	0						
	1 (469)		Ostbote r	74	**					
	1		Essex R_1	71		**	d			
	0 + 1		Ostbote r	46			**	49	94	
	0 + 1		Essex R_1	43			**	36	119	
8	0 (503)	10,000	Cobbler r	376	a	b	c			
	0		10-5-12 R_4	2						
	4 (529)	10,000	Cobbler r	285	a					
	4		10-5-12 R_4	175		**	d			
	0 + 4		Cobbler r	164			c	330	50	
	0 + 4		10-5-12 R_4	24			**	88	27	
9	0 (522)	9,500	Cobbler r	248	a	b	c			
	0		3 WM-19 $R_{1,4}$	0						
	1,4 (528)		Cobbler r	140	**					
	1,4		3 WM-19 $R_{1,4}$	150		*	d			
	0 + 1,4		Cobbler r	118			**	194	61	
	0 + 1,4		3 WM-19 $R_{1,4}$	70			**	75	93	
10	0 (503)	10,000	Cobbler r	76	a	b	c			
	0		3XX-4 $R_{2,4}$	0						
	2,4 (593)		Cobbler r	20	**					
	2,4		3XX-4 $R_{2,4}$	13		**	d			
	0 + 2,4		Cobbler r	32			**	48	67	
	0 + 2,4		3XX-4 $R_{2,4}$	10			d	7	143	
11	0 (481)	12,000	Cobbler r	15	a	b	c			
	0		3XX-1 $R_{1,2,4}$	0						
	1,2,4 (498)		Cobbler r	84	**					
	1,2,4		3XX-1 $R_{1,2,4}$	148		**	d			
	0 + 1,2,4		Cobbler r	44			**	50	88	
	0 + 1,2,4		3XX-1 $R_{1,2,4}$	61			**	71	86	

TABLE 2—Con't.

[a] Mean lesions/plant for 6 plants.

[b] Significant differences determined by Student-Newman-Keuls multiple range test. Total lesions/plant converted to logarithms for analysis. Two letters in same column in a test indicate no significant difference; asterisks below letter indicate significant differences between figures which they follow.

[c] Theoretical infection by mixtures on r hosts is the sum of infections by each race alone in tests 1-5, where the mixtures contained the same number of sporangia as the suspensions of individual races. The theoretical infection by mixtures in tests 6-11 inclusive is ½ of the sum of infection on the r host by the individual races. Theoretical infection by mixtures of clones with R genes is, of course, equal to the infection by the corresponding race in tests 1-5, and half of that amount in tests 6-11.

The reduced infection by mixtures indicates some sort of antagonism between the sporangia of the different races. For example, in tests 1-5 infection of the R variety (Cherokee) by the mixture should equal that by the specialized race alone on the same variety, because the number of sporangia of that race was the same in both suspensions. Actually, the presence of race 0 in the mixture reduced infection to 23-55% of that by the specialized race alone. All but one of these differences were statistically significant.

In the remaining tests, the theoretical infection of the R varieties should have been half that caused by the specialized race alone on the same variety. In 1 test, it was 27% of the expected, but the rest were 60-143% of the theoretical. Evidently, the antagonistic effect is greater at higher sporangial concentrations.

Similarly, infection of Cobbler or other r varieties by the mixture was 55% or less of the theoretical in the first 5 tests, but higher in some of the last 6. Apparently, the 0 isolates as well as those of the other races were suppressed in the mixtures, because in tests 2-5, infection was actually less from the mixture than from the 0 isolate alone. Again, this inhibition seems most evident when the total sporangial concentration is doubled, because in the last 6 tests, with all concentrations the same, infection of the r varieties was not inhibited as much as in the first 5 tests.

Effect of filtrates on sporangial germination.—One possible explanation for the apparent antagonism between races in mixtures is suggested by experiments in the laboratory showing that germinating sporangia produced a filterable substance that inhibited germination (zoospore production) of sporangia of the same isolate and race that produced the substance, as well as those of other races.

Several workers have observed that spore germination is inhibited in concentrated spore suspensions of various fungi (2, 7, 8, 9, 18, 30). While this inhibition may partly be responsible for relative survival ability of races in mixtures, error in experiments with concentrated spore suspension mixtures might result if other factors are obscured.

All filtrates were prepared with sporangia collected either from sporulating lesions on potato leaves or from the fungus growing on autoclaved yellow peas. The suspensions of sporangia were made with distilled demineralized water, and the sporangia/ml in 10 samples were counted with a haemocytometer. The suspensions were incubated 16-18 hours at 19-21°C to simulate the conditions of a greenhouse inoculation. Considerable direct (germ tubes) germination occurred during incubation. Indirect germination (zoospores) was not observed.

The suspensions were filtered through a sterile Seitz filter and the filtrate examined with a microscope to determine if sporangia were present. One 3-mm loop of the filtrate was transferred to each of 2 tubes of Difco nutrient broth to determine whether bacteria were present. All filtrates were sterile except that of race 4, which was contaminated with bacteria. The filtrates were stored at 2-5°C.

Fresh suspensions of sporangia from sporulating lesions either on leaves or from yellow pea media were prepared in sterile distilled demineralized water. Three ml of each sporangial suspension were added to 3 ml of the filtrate in duplicate Syracuse dishes. As a control, 3 ml of each sporangial suspension were added to 3 ml of sterile distilled demineralized water. Sporangial suspensions from 6 different races were used in all tests. The dishes were incubated 3 hours at 10-13°C.

After incubation, drops of the suspensions were placed on slides and examined with the microscope to determine the indirect germination. Two separate counts of 100 sporangia each were made from each dish.

The results (Table 3) support the following conclusions:

1) Indirect sporangial germination of all races was reduced (compared with the control) by filtrates of all races, except that the filtrate of race 2,4 had no appreciable effect on the germination of sporangia from races 0 and 1.

2) It seems probable that inhibition is caused by a substance produced by the germinating sporangia of all races, but more by some than by others. The effect is apparently nonspecific, except in the experiment with a filtrate of race 2,4, where the filtrate reduced germination of sporangia of all races with a "4" in them, but not of races 0 and 1.

Four additional experiments (not included in Table 3) with filtrates of race 0 gave essentially the same results.

Field experiments on aggressiveness and survival ability.—There are many observations, but almost no experimental evidence, on the relative survival ability of races of *P. infestans* in the field. Thurston, *et al.* (27) reported that when identical plots 400 ft apart were inoculated with race 0 and race 1, the spread of the fungus was much faster in the plot inoculated with race 0, although weather records (temperature and humidity) in the 2 plots were nearly identical. In Minnesota, isolations made in the field from varieties

TABLE 3. Percentage of indirect germination of sporangia (expressed as percentage of control) of 6 races of *Phytophthora infestans* added to filtrates of races 0; 1; 4; 1,4; and 2,4; and to water controls in 6 experiments

Filtrate source		% germination/race					
Race	No. of sporangia/ml	0	1	4	1,4	2,4	1,2,4
0	27,000	30	46	48	61	34	31
1	18,000	43	64	0	44		
4	26,000	53	61	47	54	27	6
1,4	40,000	19	29	10	21	33	
2,4	23,000	98	103	29	70	67	33
1,2,4	11,500	11	8	14	48	25	11

susceptible to all races yielded race 0 (10, 23), indicating that it is the predominant race on susceptible varieties in nature in Minnesota.

Recent work in Mexico, Canada, and the United States indicates that races other than race 0 are becoming prevalent in the field. Niederhauser, *et al.* (19) identified 15 races in 90 isolates of *P. infestans* taken from resistant potato seedlings in the Toluca Valley, Mexico. Graham (13) identified 71 isolates of *P. infestans* collected from 9 provinces of Canada in 1952-53; 57 of them were race 0, race 4, or a mixture of races 0 and 4, whereas 14 isolates were identified as race 1; 1,2; 1,4; or 1,2,4. He found that race 0 was constantly associated with race 4. Thurston (23) and Pristou and Gallegly (20) also observed that race 0 is constantly associated with race 4. It therefore appears that the majority of Graham's isolates were of the race 0 and 4 complex.

Webb and Bonde (29) made isolations from plants in 15 potato dumps in Maine and of the 11 races identified found only 1 isolate of race 0 and 1 of race 4. Fourteen isolates were from susceptible varieties, and only 1 from a resistant variety. They believed that resistant varieties had been less adequately sprayed than susceptible varieties during the previous season; and that the specialized races increased on the resistant varieties and were carried over winter in infected tubers of both resistant and nonresistant varieties.

Howatt (14) identified 1,127 isolates during studies in 1954-56 in Canada. Fourteen different races were identified from these isolates. Only 4 isolates were of race 0, but 394 were of race 4 (constantly found in association with race 0). The next largest number of isolates (211) was of race 1,4, and the remainder was of other races. The greatest number of the more complex races was found in eastern Canada, where resistant varieties are common.

Conclusions from these studies on the survival ability of race 0 compared with that of other races is difficult because: first, race 0 cannot be isolated from a resistant variety, yet many of the isolates reported in these studies came from resistant varieties; and second, the samples in most cases were small. Nevertheless, certain conclusions can be made. First, races other than 0 can multiply on varieties susceptible to all races; and second, race 0 has not been displaced

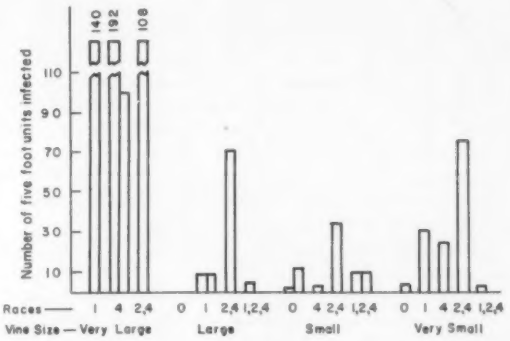

Fig. 1. Infection caused by different races on August 13 in plots classified by vine size.

in the field by the more specialized races which are present.

Differential disease spread of races 1 and 4 in the field.—During the summer of 1957, a plot 19 rows wide x 125 ft long was planted with Cobbler (r). At regular space intervals, eight 4-hill units of Essex (R_1) and 10-5-12 (R_4) were planted near either end of the plot. The plot was later inoculated with races to which Essex and 10-5-12 are susceptible. It was expected that these clones would be infected as heavily as Cobbler (assuming no difference in their field resistance—which is not known). Race 1 (isolate 469) and race 4 (isolate 470) were used separately to inoculate 2 Cobbler plants in the center of the plot. These isolates had been tested for pathogenicity and racial identity shortly before use. Notes on disease spread and defoliation were taken on 5-ft sections of each row.

Infection resulted from the initial inoculation; 9 days later the first disease spread was noted. The first disease spread was on the plants originally inoculated. Subsequently, 4 additional disease spreads occurred before defoliation became so extensive that it was impossible to differentiate discrete disease spreads.

The pattern of the second disease spread indicated that it originated from the center of the plot, where the original inoculation was made, since only plants contiguous to those inoculated were infected. The third disease spread extended to the margins of the plot; however, many of the Essex and 10-5-12 plants were not infected even though there were many lesions on the surrounding Cobbler plants. In the fourth and fifth disease spreads, this again was true, even though infection was medium to heavy in many of the surrounding Cobbler plants. Later, when Cobbler potatoes were about 75% defoliated, there was a trace of infection on Essex and 10-5-12. Finally, when the Cobbler plants were dead, Essex and 10-5-12 were 25% defoliated.

Because Essex and Cobbler were not as heavily infected as Cobbler, isolations were made from Cobbler to determine what races were present and thus possibly to help explain the anomalous results. Infected

leaves were collected at random from Cobbler, shortly after the fifth disease spread, and 26 days thereafter. After washing infected leaf tissue in running tap water and surface-sterilizing with sodium hypochlorite, isolations were made by placing sections on lima-bean agar. From several hundred isolations, 23 pure cultures were obtained. Series of differential hosts were inoculated in the greenhouse with these isolates to determine their racial identity. Of the 23 isolates, 20 were race 0; 2, race 4; and 1, race 1.

These results show that, although the plot was inoculated with races 1 and 4, race 0 composed the majority of the population of the fungus in the plot.

Differential disease spread of races 0; 1; 4; 2,4; and 1,2,4 in the field.—A field of the Cobbler variety consisting of 61 rows 420 ft long was divided into 20 plots of 19 rows (66 ft x 60 ft long). On July 22, 1957, sporangial suspensions of 5 races (0; 1; 4; 2,4; and 1,2,4) were used to inoculate the plots. Each race was used separately to inoculate 4 previously randomized plots. Two plants in the center row of each plot were inoculated. Notes on disease spread and defoliation were taken on 5-ft sections of each row.

The initial inoculation was successful. On August 9, the first disease spread was observed. The second disease spread was observed on August 13, and a third on August 16. Results of the latter were valueless for differentiating between races because blight was general over the field.

By the time of the first disease spread, there were striking differences in vine size throughout the field, which undoubtedly influenced disease spread. In 2 locations, the vines were very large, completely closing the spaces between the rows (it is probable that straw or manure piles had once been in these 2 locations). Notes were taken on size and density of vines, and plots were classified by vine size. (Plots were given the largest plant-size classification occurring in the plot if over 30 five-ft row units of that size occurred near the center of that plot.)

The 3 races infected many more row units in plots with primarily very large plants than in any other vine classification. It is doubtful that any valid differentiation can be made between the aggressiveness of the races (1; 4; and 2,4) in the plots with very large plants.

The remaining data indicate differences between the aggressiveness of races. First, race 2,4 spread most rapidly of the 5 races in all cases. Race 1 was second in rate of spread. It is difficult to rank the aggressiveness of the other 3 races (0; 4; and 1,2,4), but obviously they were not all highly aggressive.

Races 2,4; 1; and 4 all caused more infection in the plots with very small vines than in those with large and small vines. The reason for this is not known, but 1 possible explanation is that these plots were on low ground with poor drainage and that humidity was therefore higher with consequent better conditions for sporulation by the fungus.

These results indicate: 1) that the aggressiveness of races differed in the field; 2) that the isolates of races 2,4 and 1 were more aggressive under the conditions of this experiment than races 4; 1,2,4; and 0; and 3) that the microenvironment was a more important factor in the spread of the fungus than the race.

DISCUSSION.—Several criteria have been used to judge the relative aggressiveness of pathogenic races of *P. infestans:* 1) differential survival in mixtures; 2) relative rates of disease spread in experimental plots; 3) frequency of race 0 in commercial fields and the relative absence of other races; and 4) relative rates of sporulation, germination, and infection.

It is difficult to interpret greenhouse studies with mixtures of races. In such experiments, the numbers of sporangia deposited on the plants probably greatly exceed the numbers that occur in nature. Knutson (16) showed that within certain limits of concentration there is a linear relationship between sporangial concentrations and infections. At high concentrations, the number of infections/100 sporangia declined sharply. If at high concentrations race 0 causes more infection than another race, it does not necessarily follow that competition will be similar in the field. Nevertheless, in the laboratory and greenhouse studies reported, a definite antagonistic effect seemed to exist.

The fluctuation in the proportion of the 2 races from generation to generation noted in experiment 2 indicates a possible effect of environment (the nature and limits of which are not known.) A single duplicate test at 2 temperatures (Table 1) revealed very little difference in the final proportions of the races in the mixture.

The greenhouse experiments with mixtures agreed in general with field observations that race 0 is more aggressive than races with a wider varietal host range. The greenhouse experiments indicated that race 0 had superior survival in mixtures and caused more infection when plants were inoculated with similar concentrations of individual races.

There were exceptions, however; these suggest that isolates of a pathogenic race differ in aggressiveness. Unfortunately, direct comparison of isolates of a single pathogenic race is impossible in a mixture because they cannot be distinguished. The apparent differences among isolates of a race also appeared in the field tests; in 1, race 0 was very aggressive; in the other, it was least so. Also, Knutson (16) and others have shown that, when studied separately, such isolates may differ in rate of sporulation, germination, and infection.

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SEROLOGICAL STUDIES OF RED NODE AND POD MOTTLE VIRUSES

H. A. Scott, Monroe Vincent, and W. J. Zaumeyer

Respectively, Crops Research Division, ARS, U.S.D.A., Beltsville, Maryland; Microbiological Research Foundation, Bethesda, Maryland; and Crops Research Division, Beltsville.

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SUMMARY

Pod mottle and red node viruses were partially purified by a modification of the chloroform-butanol method. Antisera were prepared against both viruses. Reciprocal tests with the Ouchterlony gel-diffusion technique showed pod mottle and red node viruses were unrelated serologically and unrelated to alfalfa mosaic, southern bean mosaic, tobacco ringspot, and tobacco necrosis viruses. Red node virus is related serologically to tobacco streak virus. A virus isolate from symptomless alfalfa, previously identified as a strain of tobacco streak, is serologically related to alfalfa mosaic virus.

Introduction.—Zaumeyer and Thomas (9) described pod mottle virus (AC 95, Catalogue and Registry of the Plant Viruses, 2nd Edition, American Type Culture Collection, Washington, D.C.) as a new virus in 1948 on the basis of host range and such properties as thermal inactivation and dilution end point. Using the same criteria, Thomas and Zaumeyer later (7) identified red node virus (AC 92) as a strain of tobacco streak. Recently, Zaumeyer (8) reported that 4 of 5 virus isolates from symptomless alfalfa

resembled tobacco streak virus and tentatively characterized the isolates as a new strain of this virus. Because the serological relationships of these viruses had not been determined, investigations were initiated to develop antisera against red node and pod mottle viruses; and, through application of the Ouchterlony technique (4), to determine their serological relationships with some of the other viruses that infect beans.

In the Ouchterlony gel-diffusion technique, antigen and antibody are placed in reservoirs cut in agar. Diffusion occurs and the reactants meet in the agar and form a precipitate. One band is formed for each antigen-antibody system present. By choice of the proper pattern of reservoirs, it can be established whether 2 antigens are serologically identical, related, or different, respectively, on the basis of coalescing, partial merging, or crossing of the bands formed by the 2 antigens (3, 4). The antigen-antibody ratio affects band position and stability. A large excess of either component results in the displacement of the band away from the excessive component, and band dissociation occurs, evidenced either by a diffuse band or band-splitting (2). Selection of the proper antigen-antibody ratio results in a sharp, stable band, more or less centrally located between the reservoirs.

MATERIALS AND METHODS.—*Preparation of antigens for injection.*—Pod mottle and red node viruses were partially purified by means of a chloroform-butanol extraction (6) and alternate high- and low-speed centrifugation procedure. Bean plants (*Phaseolus vulgaris* L. var. Pinto), infected with red node virus (RNV) for 7-10 days, were harvested and disintegrated in 2 cc chloroform, 2 cc butanol, and 1 cc 0.2 *M* phosphate buffer, pH 7.0/g of tissue. The mixture was held at room temperature (*ca.* 23°C) for about 18 hours and then centrifuged at 1,200 *g* for 5 minutes. The aqueous phase was clarified at 5,500 *g* for 20 minutes and then subjected to 80,000 *g* for 90 minutes. The resulting pellets were suspended in .01 *M* phosphate buffer, pH 7.0; clarified at 2,500 *g* for 20 minutes; and kept at 4°C until injected into rabbits.

The same procedure was used for the purification of pod mottle virus (PMV), except that primary leaves of Pinto showing local lesions 5-6 days after inoculation were used as the virus source and the virus was given 3 high- and low-speed cycles. This virus does not become systemic in Pinto bean.

Both viruses were active in this partially purified form after storage for 30 days at 4°C and served as antigens for the injection of rabbits.

Immunization of rabbits.—The rabbits were immunized by 4 weekly intramuscular injections of an emulsion of equal parts of a single antigen and Freund's incomplete adjuvant from the Difco Laboratories, Detroit, Mich. Each animal received a total of 30 mg of virus preparation. The animals were bled initially during the eighth week after the injection schedule was begun and the antisera obtained were used in the present work.

Preparation of antigens for serological tests.—Alfalfa mosaic virus (AMV), tobacco necrosis virus (TNV), southern bean mosaic virus (SBMV), tobacco streak virus (TSV), and tobacco ringspot virus (TRSV) were purified partially; concentrated in the same manner as pod mottle virus; and used in testing for possible serological relationships. Table 1 lists the viruses studied, the hosts from which these were extracted, and the plants used for activity tests. Each preparation was infective. Healthy plants of the same age as infected ones were subjected to chloroform-butanol treatment and to 1 high- and low-speed cycle before being used in the serological tests. Equal amounts of healthy and virus-infected material were used.

In some serological tests, antigens from crude sap were used. Infected and healthy plants were ground in a mortar and the sap expressed through cheesecloth. The sap was clarified by centrifuging at 5,500 *g* for 10 minutes.

Preparation of Ouchterlony gel-precipitin plates.—Felsen's quadrant petri dishes were coated with 0.1% solution of agar in distilled water and dried. One g of purified agar (Difco) and 0.85 g of NaCl were added

TABLE 1. Summary of hosts which served as sources of the viruses used in serological tests, plants used for testing the activity of the purified preparations, and sources of antisera

Virus	AC no.	Source plant	Length of time of infection (days)	Host used for activity tests	Source of antiserum
Alfalfa mosaic	108	*Nicotiana tabacum* L. (Samsun)	10	*P. vulgaris* L. (Pinto)	Microbiological Associates[a]
Tobacco ringspot	98	*N. tabacum* L. (Samsun)	10	*P. vulgaris* L. (Pinto)	Microbiological Associates
Southern bean mosaic[b]	17	*Phaseolus vulgaris* L. (Black Valentine)	42	*P. vulgaris* L. (Pinto)	Microbiological Associates
Tobacco necrosis	—	*N. tabacum* L. (Samsun)	5-6	*N. tabacum* L. (Samsun)	R. P. Kahn[c]
Tobacco streak	44	*N. tabacum* L. (Samsun)	4-7	*Cyamopsis tetragonalobus* (L.) Taub.	None

[a]Bethesda, Md. Antiserum produced in cooperation with The American Phytopathological Society (1).
[b]Obtained from Irving Schneider, Growth Regulator and Antibiotic Investigations, Beltsville, Md.
[c]Plant Introduction Station, Glenn Dale, Md.

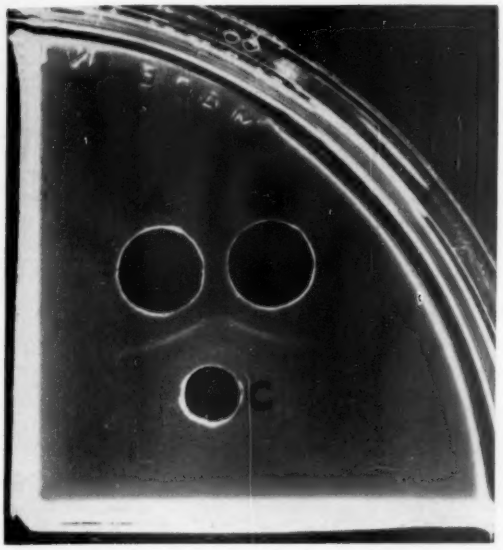

Fig. 1. Agar gel-diffusion plate showing the reaction of **A)** RNV and **B)** TSV with **C)** RNV antiserum. Source of virus was clarified sap from infected tobacco.

to 100 cc of 0.01 *M* phosphate buffer, pH 7.0; the mixture was autoclaved at 15 lb for 20 minutes. Merthiolate was added to give a final concentration of 1:10,000. The agar was added in 4-ml portions to each quadrant of the petri dish and allowed to solidify. The reservoir holes in which the sera were placed were cut with a No. 2 cork borer; those used for the antigen preparations were cut with a No. 4 cork borer. Agar plugs were removed with a pipette attached to a vacuum line. The capacities of the small and the large holes were approximately 0.03 and 0.08 cc, respectively. The 3 reservoir patterns used are illustrated in Fig. 1, 2, 3. The plates were stored at 4°C until used. After the antigens and antisera had been placed in the reservoirs, the plates were kept in moist chambers at room temperature and examined daily. The sources of the antisera are listed in Table 1.

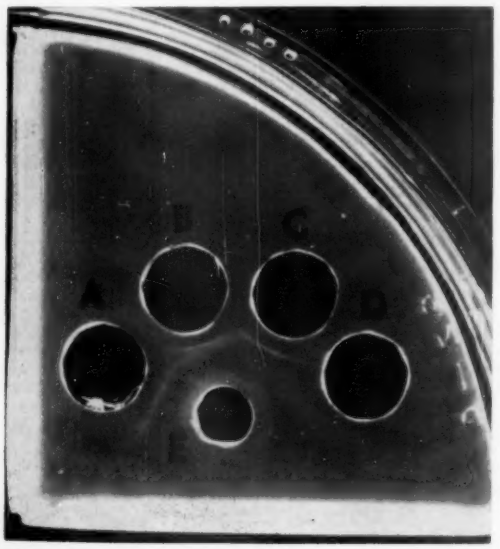

Fig. 2. Agar gel-diffusion plate showing serological relationship between **B)** RNV extracted from infected cucumber, and **C)** TSV extracted from infected tobacco. **A)** Reservoir containing extract from healthy cucumber. **D)** Reservoir containing extract from healthy tobacco. **E)** Reservoir containing RNV antiserum.

Preliminary tests were made with all the viruses and their homologous antisera by using 10-fold dilutions of the reactants. In these tests, tobacco streak virus was substituted for red node virus for reasons given below. Each virus acted as a single-membered antigenic system and obeyed the basic precepts of the Ouchterlony technique as far as band behavior was concerned. Precipitin bands were obtained most rapidly with undiluted antiserum. Therefore, antigens were tested against undiluted homologous antisera and were concentrated until sharp stable bands were obtained. When these were obtained, the antigen preparation was used in reciprocal tests with other viruses and antisera.

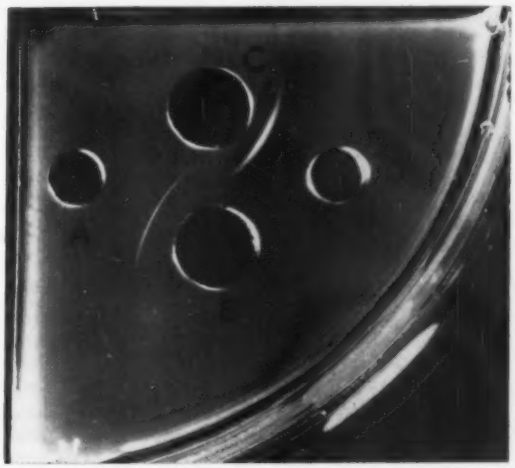

Fig. 3. Agar gel-diffusion plate showing simultaneous reaction of SBMV (reservoir **B**) and PMV (reservoir **C**) with their homologous antisera (reservoirs **A** and **D**, respectively).

Results.—Antisera against both PMV and RNV were produced. Preliminary tests were made with the antisera using the microprecipitin technique (5). The dilution end point of the PMV antiserum tested against clarified sap from infected Black Valentine bean plants was 1:1,024. No reaction with healthy bean sap was discernible. The titer of the RNV antiserum was 1:32 when tested against clarified sap of infected *Nicotiana tabacum* L. (T.I. 448A). When the RNV antiserum was tested against preparations of Pinto bean infected with RNV, the results were obscured by precipitation

due to a high titer of antibodies to normal bean protein. This complication, however, was circumvented by using infected tobacco to determine the titer of the antiserum.

Three-six coalescing precipitin bands were obtained with RNV antiserum when the antiserum was tested against clarified sap from healthy Pinto bean and RNV-infected Pinto bean in the type of Ouchterlony pattern illustrated in Fig. 1. An individual band from diseased bean, the "virus band," could not be found. When the same materials were subjected to identical chloroform-butanol extraction procedure and centrifugation, 3 or 4 coalescing bands were found and occasionally an additional band from the reservoir containing the extract from diseased plants. The large number of healthy antigen bands that interfered with viewing the reaction and the low titer of virus antigen from bean made interpretation of the results difficult. Therefore, cucumber seedlings (*Cucumis sativus* L. var. Marketer), which are susceptible to RNV, were tested as a source of antigen. Clarified sap extracted from healthy cucumber and from cucumbers infected for 6-9 days gave 3 or 4 diffuse bands, showing a serological relationship between normal cucumber and bean proteins, and occasionally a "virus band" in front of the diseased sap reservoir. When subjected to chloroform-butanol treatment and 1 high-speed centrifugation, 1 or 2 diffuse bands resulted; and a sharp band, attributed to the virus, appeared (Fig. 2-A, B).

The serological relationship between TSV and RNV was first demonstrated with clarified sap from *N. tabacum* L. (T.I. 448A), infected with each of these viruses for 7 days (Fig. 1). Fig. 2 shows the reactions of TSV from *N. tabacum* L. var. Samsun and RNV from cucumber with RNV antiserum. The fused segment of the bands is partially obscured by the diffuse band from normal cucumber antigen. In later tests by varying the antigen-antibody ratios, the "virus bands" were separated from the normal antigen bands. Since the bands formed by the 2 viruses coalesced, it was concluded that all of the structural groupings of RNV against which antibody was formed are also present in TSV (3). The materials in the antigen reservoirs had been subjected to chloroform-butanol treatment. No band occurred between the antiserum reservoir and reservoir D which contained a chloroform-butanol extract from healthy Samsun. Since tobacco is more difficult to infect with RNV than with TSV (7), tobacco infected with the latter virus was used as the source for investigations of serological relationships with other viruses.

Reciprocal tests were run between partially purified AMV, SBMV, TRSV, TNV, PMV, and TSV using the reservoir pattern shown in Fig. 3. This pattern allows the simultaneous comparison of 2 antigens and their antisera. Each virus formed a single precipitin band and no serological relationship was found among the viruses. Normal serum controls were included in each set of experiments and no bands were formed.

All tests were repeated with the type of reservoir pattern shown in Fig. 2. Two reservoirs were filled with each of 2 purified virus preparations; the 2 remaining reservoirs received preparations from healthy plants of the same type as those from which the viruses were extracted. An antiserum homologous to 1 of the viruses was tested against the various components. In addition to the RNV antiserum mentioned previously, only the PMV and the SBMV antisera contained demonstrable antibody against normal protein, but the single band which appeared was extremely diffuse and could not be found in all tests.

The virus isolate from symptomless alfalfa from Idaho which was considered related to tobacco streak virus was serologically related to AMV in gel-diffusion tests. Used in these experiments were clarified sap extracted from 3 sets of Samsun tobacco plants that had been infected for 10 days with AMV, TSV, and the Idaho isolate; and chloroform-butanol purified materials from these 3 sets. The viruses were tested against AMV and RNV antisera in the type of pattern illustrated in Fig. 3. The Idaho isolate reacted only with AMV antiserum and formed a band which coalesced with the AMV band.

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EVIDENCE FOR FUNCTIONAL CYTOCHROME OXIDASE IN TISSUES INFECTED BY OBLIGATE PARASITES

J. M. Daly and S. G. Jensen

Plant Pathology Department, University of Nebraska, Lincoln.

Plant Pathologist and Research Assistant.

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SUMMARY

Three compounds (sodium azide, sodium diethyldithiocarbamate, and carbon monoxide), used as inhibitors of oxidases, were tested for their effect on respiration of intact tissue during development of rust diseases of safflower, bean, and wheat. Healthy and diseased safflower respiration was inhibited strongly by azide at all stages tested, suggesting participation of heavy-metal oxidases in respiration. Inhibition by diethyldithiocarbamate was variable. CO consistently inhibited respiration of rusted hypocotyls at the time of maximum respiration induced by disease. CO inhibition was reversed by light in manometric and mass spectrometer experiments. Thus, at a minimum, 40% of the respiration of rusted safflower hypocotyl respiration was mediated by cytochrome oxidase. The effect of CO was not so pronounced on healthy hypocotyls of the same age.

Respiration of rust-affected leaves of wheat and bean also was inhibited by CO, suggesting operation of cytochrome or polyphenol oxidase rather than ascorbic acid oxidase. Since CO stimulated respiration in healthy leaves of these species, identification of cytochrome oxidase by light reversal was not attempted in mass spectrometer experiments.

INTRODUCTION.—There has been a recent increase in interest in the nature of the enzymes responsible for electron transfer in diseased plant tissue (10, 11, 14, 15, 20). Much of the literature has been reviewed by Farkas and Király (11) and more recently by Heitefuss, Stahmann, and Walker (15). Ascorbic acid oxidase and polyphenol oxidase have been the 2 main oxidase enzyme systems reported in disease caused by facultative parasites (11, 15, 20, 25). In cases of obligate fungus parasitism, Király and Farkas (19) have reported that nearly all of the additional respiration of wheat caused by infection with *Puccinia graminis tritici* Eriks. & Henn. is mediated by ascorbic acid oxidase whereas a decrease in glycolic acid oxidase occurs (18). It is surprising that cytochrome oxidase has not been reported functional in diseased tissue since recent, more careful appraisal of this oxidase has demonstrated its importance in a wide number of higher plants and microorganisms (2, 6, 24). Hassebrauk and Kaul (13) obtained evidence that cytochrome and ascorbic acid oxidases were at high levels in uninfected wheat varieties resistant to *P. triticina*.

Although there is evidence that ascorbic acid and polyphenol oxidases may be involved in symptom development (20) or expressions of disease resistance (25), proof of such functions is difficult to obtain. Most frequently, conclusions are based on the catalytic activity of homogenates on various substrates with supporting studies of inhibitors and substrates on intact tissue. The homogenate technique is difficult to interpret because in a single species several oxidases may be present, each capable of supporting the total respiratory rate *in vivo* (1, 6, 14, 16, 21, 24). The use of inhibitors and substrates on intact tissue also has limitations because of a lack of specificity and problems in uptake. Kinetic complexities arise from possible shifts in oxidase activity to a second system in the presence of an inhibitor. Possible difficulties in connection with studies of obligate parasitism are indicated in reports on the potential importance of cytochrome oxidase (27) and polyphenol oxidase (12) in spores of *P. graminis tritici*.

The present work was initiated to determine whether CO inhibition might be useful as a criterion to establish the nature of the oxidase systems in disease caused by rust fungi. In contrast to organic inhibitors, this gas diffuses readily to oxidative sites. When properly employed, it can readily distinguish ascorbic acid, polyphenol, and cytochrome oxidase. The former is unaffected and although both the latter are inhibited, cytochrome oxidase inhibition is readily reversed by light. In addition, the extent of heavy metal enzyme participation was studied using azide as an inhibitor; diethyldithiocarbamate (Dieca) was employed because of its supposed effectiveness as an inhibitor of ascorbic acid oxidase (17).

MATERIALS AND METHODS.—Growth and inoculation of host plants were carried out by procedures described previously (5, 7). For respiratory measurements, normal and heavily infected safflower hypocotyls were sectioned to 2-3 mm, washed with distilled water, and individual 500-mg samples weighed. With bean and wheat, leaf sections of approximately 25 mm^2 were cut from primary leaves.

In experiments in which azide ($5 \times 10^{-4}M$), Dieca

($2 \times 10^{-4}M$), and CO were compared, the following procedure was used. Immediately after weighing all samples, Dieca treatments were initiated by placing the tissues in 500 ml of freshly prepared $2 \times 10^{-4}M$ Dieca in 0.1 M KH_2PO_4 (pH 4.6) in the dark. The Dieca solutions were equilibrated and maintained at the same temperature at which respiration rates were measured. Tissues were rinsed in distilled water and transferred to fresh solutions of Dieca at the end of 15, 30, and 45 minutes. At 60 minutes, the tissue was rinsed and placed in Warburg flasks containing 2 ml of 0.1 M KH_2PO_4, but no Dieca. Pretreatment procedures are necessary since, as originally determined by James and Garton (17), Dieca spontaneously decomposes to yield gaseous products. The frequent changes ensure adequate penetration of intact inhibitor molecules. Use of Dieca during respiratory measurement was avoided since decomposition in Warburg vessels would result in spurious pressure changes.

Tissue for CO studies was placed in 0.1 M KH_2PO_4 in the Warburg apparatus and 2 liters of a mixture of 80% CO and 20% O_2 were flushed slowly through the vessels during shaking. Other samples were placed in either 0.1 M phosphate buffer or phosphate buffer containing $5 \times 10^{-4}M$ sodium azide.

At the end of the Dieca treatment, all vessels were given an additional 10 minutes of thermal equilibration at 18°C. Respiratory rates were determined at 10-minute intervals for at least 1 hour in the dark. The above procedure standardized time of exposure to inhibitors and permitted ready comparisons at different stages of rust development.

In some manometric experiments, the effect of light on CO inhibition was determined by 0.5-hour exposure of tissues to fluorescent light of 40 ft-c. A circular fluorescent tube was placed 6 in. below the vessels on the bath. Rates were compared with rates obtained in the dark for 0.5 hour before and after exposure to light.

The mass spectrometer measurements were made in the Department of Botany, University of Minnesota, with the apparatus developed by Brown and coworkers (6, 25). Sections of moistened safflower hypocotyls were placed in rectangular vessels without a liquid bathing medium. Standard gas mixtures of CO_2, O_2, and either N_2 or CO were flushed into the system. A small section of the circulating gas phase was evacuated and isotopic O_2 of masses 32 and 34 introduced. The final composition of the experimental gaseous phase was 4-5% O_2 (masses 32 and 34), 2% CO_2, and the remainder either N_2 or CO. In the experiments of Fig. 2, the N_2 studies were performed first for each type of tissue and then the effect of CO was determined. The light intensity was approximately 50 ft-c.

The partial pressure of masses 32 and 34 was recorded every 108 seconds on a Brown potentiometer. Since we were interested only in the relative rates of O_2 uptake in dark and light periods, the units of Fig. 2 are given in relative units. The partial pressures are

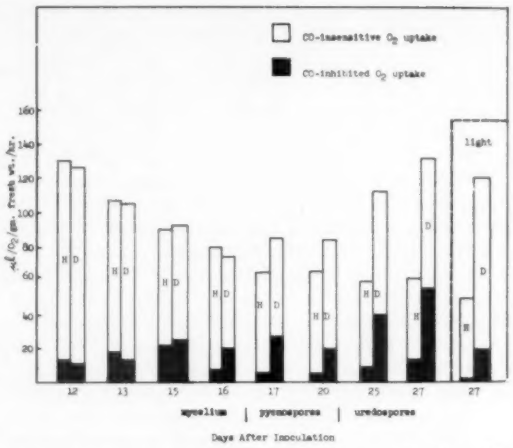


Fig. 1. The effect of CO on respiration of healthy (H) and diseased (D) hypocotyls of safflower. The total height of the bars represents O_2 consumption in the dark/g fresh weight per hour in air. The height of the dark areas represents O_2 consumption with CO/O_2 (80:20) as the gas phase. The dotted line insert indicates the effect of light for the sample of the twenty-seventh day.

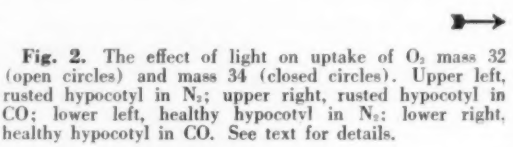
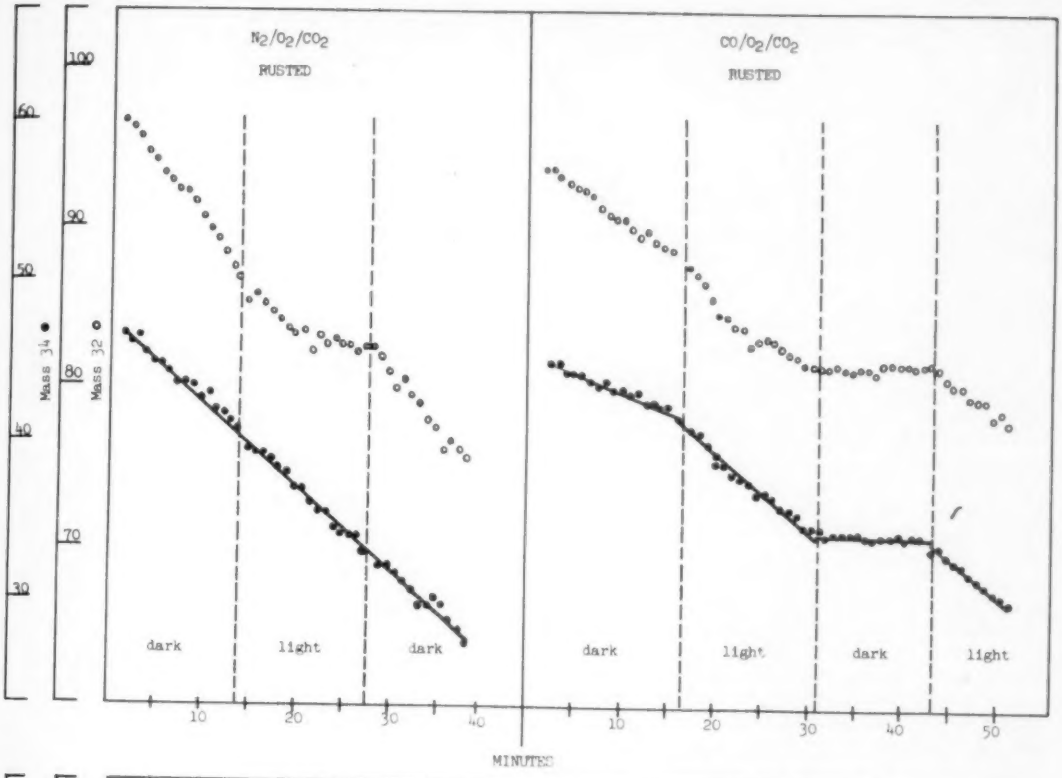


Fig. 2. The effect of light on uptake of O_2 mass 32 (open circles) and mass 34 (closed circles). Upper left, rusted hypocotyl in N_2; upper right, rusted hypocotyl in CO; lower left, healthy hypocotyl in N_2; lower right, healthy hypocotyl in CO. See text for details.

Table 1. Effect of inhibitors on the respiration of healthy (H) and rust-affected (D) safflower hypocotyls

Age (days)	Resp. rate (μl O_2/g/hr)		% inhibition: $5 \times 10^{-4}M$ Sodium azide		% inhibition: $2 \times 10^{-4}M$ Dieca		% inhibition: CO/O_2 (80:20)	
	H	D	H	D	H	D	H	D
10	221	211	69	77	28	33	39	43
13	151	155	80	88	33	44	34	44
15[a]	118	147	75	85	44	46	26	39
17	141	182	73	85	38	51	26	40
20	116	149	74	89	41	57	26	35
24[b]	84	152	71	88	59	57	14	38

[a] Pycnia evident.
[b] Urediospores evident.


$N_2/O_2/CO_2$
RUSTED
$CO/O_2/CO_2$
RUSTED
Mass 34 ●
Mass 32 ○
dark
light
dark
dark
light
dark
light
MINUTES

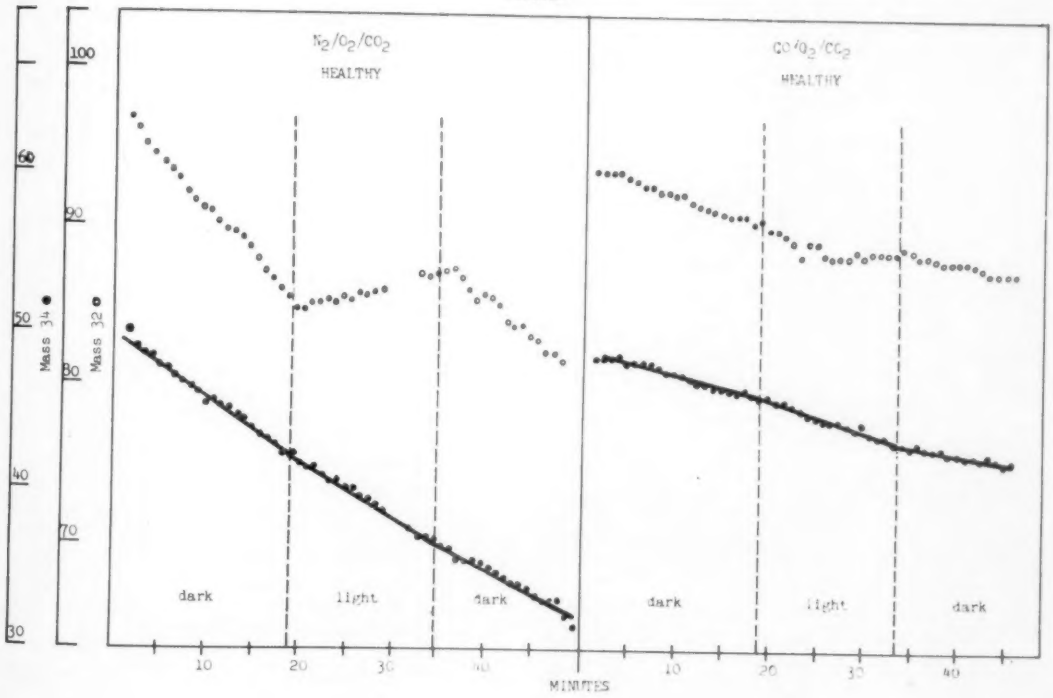

$N_2/O_2/CO_2$
HEALTHY
$CO/O_2/CO_2$
HEALTHY
Mass 34 ●
Mass 32 ○
dark
light
dark
dark
light
dark
MINUTES

TABLE 2. Effect of inhibitors on the respiration of healthy (H) and rust-affected (D) bean leaves

Days after inoculation	Resp. rate (μl O_2/g/hr)		Rate as % of controls					
			$5 \times 10^{-4}M$ Sodium azide		$2 \times 10^{-4}M$ Dieca		CO/O_2 (80:20)	
	H	D	H	D	H	D	H	D
1	189	194			74	71	110	120
3	184	209	133	115	82	79	114	115
5[a]	172	244	132	110	82	86	122	90
7[b]	152	259	148	150	89	74	119	93
11[c]	118	195	105	105	100	70	112	84

[a] Flecks.
[b] Incipient sporulation.
[c] Heavy sporulation.

recorded as distance of pen excursion of the recording potentiometer (peak height) for each mass. Weis and Brown (25) have shown that it is necessary in some experiments to make corrections for "preferential reutilization" when isotopic species of metabolic gases are measured. These corrections, involving consideration of the diffusion barriers between liquid and gas phases, are important for absolute rates of O_2 consumption at light intensities 4-5 $\times$ the compensation point. Since the photosynthetic capacity, particularly of rust-affected tissue, was low as was the light intensity, these corrections are not given in Fig. 2. Furthermore, the rates of diffusion and the nature of the diffusion barriers in large sections of multicellular tissue are difficult to measure and interpret. The primary objective of the mass spectrometer experiments was to resolve possible ambiguities in manometric data (see below).

RESULTS.—The changes in rate of respiration with development of rust were similar to those previously reported (5, 7) for safflower (Table 1, Fig. 1) and bean (Table 2).

Safflower was inhibited markedly by azide, but the degree of inhibition was not strikingly different between healthy and diseased tissue. Maximum differences were observed after the respiratory rates were higher in the rust-affected tissue. The results with Dieca were not consistent. In the data of Table 1, except for the twenty-fourth day, there was a tendency for diseased hypocotyls to be inhibited more by Dieca; however, subsequent experiments did not always show this. In 1 series, there was less inhibition of infected tissue by Dieca. Consistent and sharp differences were found when CO was used as a respiratory inhibitor. Maximum differences in percentage inhibition were observed in the later stages of disease development. In young normal safflower hypocotyls, a high proportion of the respiration was affected by CO. As the tissue aged, however, the effect became less marked. In the first experiment, the diseased tissue maintained the initial high sensitivity to CO as the respiratory rates increased (Table 1).

In a second experiment, in which both healthy and diseased tissue initially had lower rates of respiration than those of Table 1, the initial inhibition by CO of both tissues was low (Fig. 1). As the respiration rate of the infected hypocotyls increased, however, the amount of respiration sensitive to CO also increased (shaded areas of bars).

Included in Fig. 1 (dotted line insert) are data obtained on net gaseous uptake by rusted and healthy tissue in the light. A comparison of the shaded area of each bar shows that the CO inhibition of both types of tissue is at least partially reversed by light. It was observed, however, that the total O_2 consumption of healthy and diseased tissue was less in the light than in the dark. Both rusted and diseased tissue contained small amounts of chlorophyll; this suggested 4 possible limitations of the manometric measurements: 1) photosynthetic production of O_2; 2) photo-inhibition of respiration (25); 3) photo-oxidation by the tissue in the absence of CO_2 (6); 4) combinations of these possibilities.

To eliminate some of the possible explanations, experiments were conducted with a mass spectrometer (Fig. 2). Since the tissues contained only O_2 (mass 32), it is possible to follow uptake of O_2 in the light using O_2 34 as a tracer. The lower graph of Fig. 2 shows uptake by healthy tissue of O_2 32 and 34 in the dark under N_2. During the light period, mass 32 produced by photosynthesis increased. Tracer O_2 34 was removed from the gas phase at essentially a constant rate in both light and dark periods. Under CO, however, the slope of the graphs of both masses was less steep. In the case of mass 34, the slope essentially was unchanged during the light period. This also was true of mass 32, suggesting a CO inhibition of photosynthesis.

In the case of diseased tissue under N_2, the slope of the mass 34 line was not altered appreciably by light and the failure of the mass 32 partial pressure to increase as with normal tissue indicates a lower rate of photosynthesis. With CO in the gas phase, light markedly altered the rate of mass 34 uptake. This is particularly noticeable when the 2 light periods are compared with the intervening second period of darkness. In contrast to the manometric experiments, mass spectrometer readings were initiated immediately in CO/O_2 volume ratios of approximately 93:4. Since CO is a competitive inhibitor, the effect of a 20:1 ratio of inhibitor to substrate would give a greater inhibition than the ratio of 4:1 employed

manometrically. Such considerations would explain differences in slope in the first and second dark periods. The important feature is the more rapid rate of uptake of mass 34 in both light periods, with rates approximately equal in each instance.

Attempts to show a similar CO-sensitive respiratory enzyme in rust-affected primary leaves of bean and first leaves of wheat posed unexpected problems. These are illustrated in Table 2. Although Dieca produced greater inhibition in diseased tissue, the significance of the results cannot be determined easily for reasons discussed in a later section. Azide at $5 \times 10^{-4}M$ consistently stimulated respiration, even in diseased tissue where the rates in the absence of inhibitor were appreciably above the healthy controls. With CO/O_2 (80:20) healthy leaves also showed a stimulation, as did diseased tissue before respiration rates had increased. In the later stages of infection, after flecking when rates were higher, CO inhibited respiration of diseased plants.

The effect of CO was similar with wheat. In a series in which healthy wheat was tested from the earliest stages of growth when sufficient leaf material was available until 3 weeks after planting, it was found that very young leaves were inhibited by CO. About 10 days after planting, however, when the first leaf was fully expanded they showed consistent stimulation ranging 10-40% above the controls. At this point, leaves inoculated as early as possible with rust fungi show an increase in respiratory rates. Consequently, the healthy leaves were stimulated while the diseased leaves showed moderate (10-30%) inhibition by CO.

Discussion.—The data with safflower show clearly that, at a stage in disease development when respiration is at a maximum, cytochrome oxidase is a functional component of the increased rates. The data, however, are not amenable to a quantitative characterization of the proportion of the total respiration that is carried over the cytochrome system. In both healthy and diseased tissue, nearly all of the respiration is inhibited by low concentration of azide, suggesting the importance of heavy metal-containing enzymes in both types of tissue. In contrast to healthy tissue which was not appreciably affected, diseased tissue was inhibited approximately 40% at CO/O_2 ratios of 4:1 and a considerable proportion of the inhibition was reversed by light. CO is a competitive inhibitor and complete light reversal is a difficult goal to achieve in large segments of intact tissue. Thus, under normal atmospheric levels of O_2, a minimum of 40% of the respiration occurs *via* the cytochrome system. The percentage may be nearly 100%, as indicated by the complete inhibition at ratios of 20:1 in the mass spectrometer experiment. Furthermore, the light reversal results in rates equal to the rates in the absence of CO. The O_2 tensions employed, however, are physiologically low and this causes some uncertainties because of the higher affinity for O_2 by cytochrome oxidase than, for example, ascorbic acid oxidase (27). Further, healthy tissue did not show a marked light reversal under these conditions.

Increased CO-sensitivity of diseased tissue, when compared with healthy tissue at the time of maximum increased O_2 consumption during infection, may or may not represent the appearance of new oxidase systems distinct from those of the host. It has been suggested (7) that the rust mycelium in safflower hypocotyls is responsible for the increased rates. Despite a negative report (9) which is open to question, the occurrence of cytochromes in 45 species of fungi (2) and their demonstration in rust spores (26) indicate the feasibility of the suggestion. Although mature normal tissue is not markedly affected by CO, there is a small effect. Failure to demonstrate a light reversal can be explained by the lower rates of respiration (6, 23). Younger, more metabolically active tissue is more sensitive to CO. Thus, host metabolism may be involved in the host-parasite complex.

Since there is a reasonable doubt as to the extent of cytochrome oxidase participation, the inhibition with Dieca might be ascribed to ascorbic acid oxidase inhibition. It should be remembered, however, that this compound does not show complete specificity for Cu enzymes. In the original work, James and Garton (17) found $10^{-3}M$ concentration inhibited cytochrome oxidase approximately 40%. Even at $2 \times 10^{-4}M$, Dieca inhibited cytochrome oxidase 8-10% and could not distinguish between ascorbic acid and polyphenol oxidases (17). Unfortunately, this concentration and higher ones have been used in experiments purporting to show ascorbic acid oxidase activity in diseased tissue (14, 18). In addition to a lack of specificity, results obtained with Dieca are subject to possible discrepancies because of unequal uptake by tissues respiring at different rates. Greater metabolic accumulation of an organic molecule such as Dieca could occur in rusted areas as Shaw and Samborski (21) have shown for a variety of compounds. Consequently, the concentration of Dieca at the site of oxidase activity might be high enough to affect cytochrome oxidase. Greater inhibition by this compound in diseased tissue would not necessarily indicate ascorbic or polyphenolase activity.

The cytochrome system may be of considerable importance in the generation of phosphate bond energy (3). With other oxidases, the production of metabolic energy is not well documented. Cytochrome systems are most readily detected in young, actively metabolizing plant tissues, with high demands for energy in synthesis. Generation of metabolic energy during electron transport at the peak of respiratory activity could furnish pools of phosphate nucleotides required to drive the synthesis of compounds necessary for growth and sporulation of obligate parasites (7). Hackett and Schneiderman (13) have shown that a light-reversible CO inhibition of both respiration and growth occurs in higher plants, supporting the idea that electron transport by cytochromes may play a key role in synthetic reactions.

For these reasons, the identity of the terminal

oxidases in diseased tissue assumes real importance. It is tempting to correlate the presence of ascorbic acid oxidase (18) with the possible existence of an uncoupling mechanism in rusted wheat (22), or with different pathways of respiration (5, 8, 22). The evidence for ascorbic acid oxidase in rusted wheat, however, is not adequate for a final evaluation. Király and Farkas relied on 1) increased oxidation of ascorbic acid by homogenates and 2) the use of Dieca in homogenates and intact tissue. Ascorbic acid may be oxidized by the cytochrome system or may be metabolized *via* enzymatic reactions not related to catalytic oxidation. These workers concluded that cytochrome oxidase was absent because of failure of *p*-diphenylenediamine to stimulate oxygen consumption, but no tests with cytochrome C are reported. It is recognized (23) that crude homogenates contain substances inhibitory to cytochrome oxidase. Furthermore, the apparent localization of ascorbic acid oxidase in cell walls, rather than in cellular particles, again raises the question as to its functional significance in intact tissue. The work of Honda (15) illustrates the difficulties of interpreting the role of ascorbic acid oxidase *in vivo* and the limitations of Dieca as an indicator of its activity in homogenates and tissues.

The other line of evidence presented by Király and Farkas (19) is also open to question, since in Table 4 they show a 68% inhibition of respiration (inhibition of controls not given) with $10^{-3}M$ Dieca. Data in a footnote of the next table, however, permit calculation of an inhibition that is much less than that in Table 4 (30%) and far lower than the control tissue.

In contrast to their data, the results of the present study show that with rusted wheat and bean tissue there is a CO-sensitive oxidase functional during the period of maximum respiratory activity, suggesting cytochrome or polyphenol oxidase. The marked stimulation of healthy tissue in CO, however, prevents a ready test for light-reversal of the inhibition. It is probable that CO is acting as an uncoupling agent as previously suggested (4). Similar phenomena have been reported recently (23, 27), and their occurrence in bean and wheat requires other approaches to the problem of oxidase systems in rust-affected plants. Nevertheless, the presence of a CO-sensitive system in parasitized tissues emphasizes the need of re-evaluation of previous work.

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PRODUCTION OF CELL-WALL DISSOLVING ENZYMES BY CLADOSPORIUM CUCUMERINUM IN CUCUMBER TISSUE AND IN ARTIFICIAL MEDIA

D. L. Strider and N. N. Winstead

Department of Plant Pathology, North Carolina State College.

Respectively, Research Assistant Professor of Plant Pathology, Mountain Horticultural Crops Research Station, Fletcher, North Carolina; and Associate Professor of Plant Pathology, North Carolina State College.

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SUMMARY

Cladosporium cucumerinum produced cellulase (Cx), polygalacturonase (PG), and pectin methylesterase (PME) *in vitro*. Cx activity was demonstrated in filtrates from cultures containing cellulosic or noncellulosic carbon sources. Cx produced by *C. cucumerinum* was thermolabile, but a commercial cellulase was slightly active after autoclaving 1 hour at 123°C. Greatest PG activity occurred in filtrates from cultures containing citrus pectin, but some PG activity was noted in filtrates from cultures containing a number of other carbon sources. PME activity was demonstrated from cultures containing citrus pectin or sodium polypectate as carbon sources. Attempts were unsuccessful to inhibit activity of these 2 enzymes with acetic acid or cysteine.

Extracts from scab-diseased cucumber seedlings, grown aseptically, were high in Cx activity but low in PG activity. PME production by the fungus in cucumber tissue was not determined, since both healthy and diseased tissues contained this enzyme.

Since large amounts of Cx were found in diseased cucumber sap, it was concluded that Cx probably plays an important role in the disintegration of cucumber tissue by the pathogen.

INTRODUCTION.—The most obvious symptom of scab of cucumber (*Cucumis sativus* L.) is disintegration of tissues of foliage and fruit. Symptom expression and previous reports (3, 4, 5, 9) suggested that a study of the cell-wall dissolving enzymes (Cx, PG, and PME) might elucidate the destructive nature of the pathogen (*Cladosporium cucumerinum* Ell. & Arth).

REVIEW OF THE LITERATURE.—Pierson and Walker (9) studied disease development of *C. cucumerinum* in cucumber and observed direct penetration under well-defined appressoria. Mycelium in the susceptible variety was intercellular at first, becoming intracellular after invaded cells had been killed, presumably by enzymatic action. A similar pattern of disease development with susceptible tissue was reported by Fouad (3). From observations of stained, infected tissue, Fouad concluded that the fungus secreted a pectinolytic enzyme which dissolved parts of the middle lamella and changed the chemical or physical properties of cell walls.

Husain and Rich (5) studied the pectinolytic and cellulolytic enzymes produced by *C. cucumerinum, in vitro*. They detected PG and cellulase activity, but no PME activity. PG activity was demonstrated when the organism was grown on dextrose, but little PG was found when citrus pectin was the carbon source. The cellulase enzyme was considered adaptive, since large quantities were produced when various cellulosic materials were used as carbon sources, whereas only small quantities were reported on dextrose. The authors concluded that disintegration of cucumber tissue by *C. cucumerinum* was by PG and cellulase produced by the pathogen.

MATERIALS AND METHODS.—Crude enzymes were obtained as filtrates from cultures grown 6-30 days on Richard's solution at pH 4.5 modified to contain, as the carbon source, 2% of dextrose, galacturonic acid, cellobiose, carboxmethylcellulose (CMC, type 7mp, Hercules Powder Co.), filter paper pulp (Whatman No. 1), citrus pectin (Exchange, Lemon Products Co.), sodium polypectate (California Fruit Grower's Exchange), levulose, or sucrose. Filter paper, CMC, citrus pectin, and sodium polypectate were blended separately with the basal medium for 2 minutes before autoclaving. An identical series of media was prepared to which 0.1% yeast extract was added. Two other media were prepared, 1 containing 0.1%

yeast extract as a carbon source, and the other containing neither a carbon source nor yeast extract.

After growth, media were aseptically freed of the organism by filtration under vacuum through 2 layers of filter paper. These filtrates were streaked on potato-dextrose agar (PDA) and nutrient agar (NA) to check for contaminating organisms. Filtrates of commercial pectinol (Pectinol 100-D, Mix 79, Rohm & Haas) and commercial cellulase (Enzyme 19, Mix 12, Rohm & Haas) also were assayed for relative enzyme activity. Samples boiled for 10 minutes were assayed as checks.

Estimates of enzymatic activity were by the viscosity method (2, 7), except for PME, which was estimated by Smith's (13) technique. In this paper, the enzyme system which hydrolyzes the soluble cellulose derivative (CMC) is called the cellulase system (Cx) in accordance with the multi-enzymatic theory advanced by Reese (11). Changes in viscosity were measured in size 300 Oswald-Fenske viscometers in a water-bath with continuous agitation at 30°C. Substrates were 1.2% sodium polypectate, citrus pectin, or CMC, adjusted to pH 5.5 and containing 0.04 *M* sodium hydroxide and 0.02 *M* citric acid buffer. Substrate was slowly added to the warm (60°C) buffer solution while blending for 2 minutes. For uniformity, the substrate was strained through 2 layers of cheesecloth. After cooling, 4 ml of toluene/liter of substrate were added before storing at 4°C.

Each enzyme test was repeated at least 3 times. Two replicates were from filtrates of cultures prepared at 1 date and 1 replicate from filtrates of cultures prepared at a different date. Fresh commercial enzyme solutions were prepared for each test.

To determine if a polygalacturonase-type enzyme was involved in reduction in viscosity of citrus pectin, 3 ml from the filtrate of 11-day-old cultures grown on citrus pectin as a carbon source were incubated 12 hours with 15 ml of the citrus pectin substrate and analyzed for the presence of galacturonic acid by paper chromatography (16).

Since cysteine (14) and acetic acid (15) are toxic to *C. cucumerinum* and since cellulase inhibition with cysteine has been shown (1, 17), we studied the effect of cysteine and acetic acid on the activity of Cx and pectic enzymes produced by the pathogen. Crude enzymes were filtrates from cultures grown on citrus pectin or sodium polypectate as carbon sources. Methods of measuring activity were the same as above. For viscosity tests, 1 ml of 0.1 *M* cysteine or acetic acid, adjusted to pH 5.5 with NaOH, was added to 5 ml of substrate and mixed before addition of crude enzyme. One ml of sterile distilled water was added to controls. Tests for PME inhibition also were conducted.

To determine the presence of these enzymes *in vivo*, Stono cucumber plants were grown aseptically in 2-liter flasks containing coarse sand and vermiculite. Flasks were placed in the greenhouse at about 29° C; after 2 weeks, plants were inoculated by placing a drop of spore suspension on terminal growth. Incubation was at 16°C in subdued light. Plants in 2 flasks were not inoculated and served as checks. A portion of the extracted juice to be used on pectic substrates was dialyzed against distilled water. Contamination was checked on NA. Plants were grown under aseptic conditions only once, but enzyme tests from this sample of plants were performed 3 times.

TABLE 1. Percentage of loss in substrate viscosity after a 4-hour incubation with filtrates obtained from 7-day-old cultures of *C. cucumerinum* grown on various carbon sources and containing 0.1% yeast extract

Carbon source of culture	% loss in viscosity		
	Citrus pectin	Sodium polypectate	CMC
Sodium polypectate	30	27	90
Dextrose	15	30	11
Sucrose	16	16	44
Citrus pectin	95	92	0
Cellobiose	6	14	0
Galacturonic acid			7
CMC	0	0	30
Filter paper	0	0	30
Yeast extract (0.1%)	0	0	0
Commercial cellulase (100 ppm)			82
Pectinol (100 ppm)	72	95	

RESULTS.—As reported previously (14), *C. cucumerinum* grows on many different carbon sources. Addition of yeast extract greatly stimulated growth on all carbon compounds. The organism grew poorly on yeast extract as the sole carbon source. In the absence of yeast extract, the fungus failed to grow on filter paper pulp after 30 days and grew poorly on cellobiose, CMC, and galacturonic acid. Best growth occurred with sodium polypectate as the carbon source.

Filtrates from 7-day-old cultures containing the several carbon sources amended with yeast extract were examined for PG, PME, and Cx activity. Viscosity losses indicated the presence of Cx and pectic enzymes (Table 1). (Carbon sources are ranked in Table 1 according to amount of mycelium observed at harvest, from greatest to least.) Heated samples of culture filtrates failed to reduce substrate viscosity, but heated commercial cellulase samples reduced viscosity of CMC 39% after a 4-hour incubation. No PME activity was detected from 7-day-old cultures.

Cx, PG, and PME activity was studied using filtrates obtained from 9- to 21-day-old cultures lacking yeast extract. Viscosity readings were taken at intervals up to a 24-hour incubation of enzyme sample-substrate. Samples of filtrates were autoclaved 6 minutes and assayed as checks. Cx activity was present in filtrates from cultures containing sodium polypectate, sucrose, or CMC. Reduction in viscosity of CMC substrate with CMC filtrates was very slight, but considerable reduction was observed using the other 2 filtrates. For example, the sodium polypectate filtrate reduced the viscosity of CMC more than 50% after 1 hour. Activity again was noted with the heated commercial cellulase preparation, but not with heated filtrates. Viscosity of sodium polypectate substrate was reduced

by filtrates from all media, regardless of carbon source, except the filter paper medium, on which no growth occurred. Filtrates most active in reducing viscosity of sodium polypectate were from cultures containing citrus pectin. In this case, 50% loss in viscosity was recorded after a 1-hour incubation. After a 12-hour incubation, reduction in viscosity of citrus pectin was recorded only with filtrates from those cultures containing citrus pectin, sodium polypectate, sucrose, levulose, and galacturonic acid. After a 24-hour incubation, however, all filtrates reduced the viscosity of citrus pectin, except the medium containing filter paper, on which no growth was observed. The most active filtrate on citrus pectin substrate was obtained from the medium containing citrus pectin. In general, viscosity of sodium polypectate substrate was reduced to a greater degree than was citrus pectin substrate, when acted on by the same crude enzyme preparation. PME activity was recorded with filtrates from 21-day-old cultures containing either sodium polypectate or citrus pectin. Approximately 0.7 ml of 0.05 *M* NaOH was required for the color change back to blue (indicating degree of demethylation), when sodium polypectate filtrate was used; only 0.3 ml was required when citrus pectin filtrate was used.

Since boiling for 10 minutes failed to inactivate commercial cellulase, the enzyme preparation (100 ppm) was subjected to more drastic heat treatments. Five-ml portions in 30-ml test tubes were autoclaved at 123°C for various periods of time up to 60 minutes. All preparations were active, although after 60 minutes only slight reduction in viscosity of CMC could be detected. After a 9-hour incubation, at least 50% loss in viscosity of CMC was recorded with preparations autoclaved for 20 minutes.

Chromatograms revealed large amounts of galacturonic acid in citrus pectin filtrates incubated with citrus pectin substrate. No galacturonic acid spots appeared when citrus pectin filtrate was chromatographed alone; no sugar spots appeared when citrus pectin substrate was chromatographed alone. These results indicate pectic-substrate viscosity was, at least partly, reduced by PG produced by the pathogen.

The viscosity of CMC was rapidly reduced by crude enzyme preparations obtained from diseased seedlings grown under aseptic conditions prior to inoculation. More than 50% loss in viscosity was recorded after a 30-minute incubation with a 1:10 dilution of diseased cucumber sap. Extracts from nondiseased seedlings did not reduce the viscosity of CMC. Hence, the fungus produced Cx *in vivo*. Very slight loss in viscosity of citrus pectin and sodium polypectate was detected in extracts of diseased seedlings. After a 9-hour incubation, the average reduction in viscosity of citrus pectin was 17% and of sodium polypectate, 13%. No loss in viscosity of pectic substrates was observed with heated extracts or with extracts from disease-free plants. These results indicate the production of a pectic enzyme by the fungus *in vivo*. Positive tests for PME activity were obtained with extracts from diseased and disease-free cucumber. Negative results were obtained with heated extracts. Since substrate formed a gel with extracts, titration with base was not performed.

Neither cysteine nor acetic acid reduced the activity of the enzymes studied.

Discussion.—Results presented confirm those of Husain and Rich (5) on production of PG and a cellulolytic enzyme by *C. cucumerinum, in vitro*. In addition, the pathogen was shown to produce PME, *in vitro*. Cellulolytic activity was demonstrated in filtrates obtained from media in which sodium polypectate, sucrose, or CMC was supplied as a carbon source. These results do not support the contention of Husain and Rich that Cx produced by *C. cucumerinum* is adaptive in nature. Husain and Rich found large amounts of PG in filtrates from cultures containing dextrose, but little or none from cultures containing citrus pectin. In the present investigation, greatest PG activity was demonstrated from filtrates obtained from cultures containing citrus pectin as the carbon source. PG activity was further demonstrated in filtrates from cultures containing various other carbon sources. It was never possible in these investigations, with undiluted filtrates, to obtain the level of PG or Cx activity reported by Husain and Rich (5) with filtrates diluted 1:1. The heated filtrates, in almost every case, initially increased the viscosity of the substrate. This effect also was noted in many cases with nonheated filtrates. PME activity was demonstrated with filtrates obtained from cultures containing citrus pectin or sodium polypectate as carbon source. Negative tests for PME were recorded with filtrates from cultures less than 9 days old, which possibly explains the negative results of Husain and Rich in relation to production of PME, since they only used filtrates from cultures 4-7 days old. Since PME was present in healthy cucumber tissue, PME production by the pathogen *in vivo* was not demonstrated.

Failure to demonstrate high PG activity in extracts of diseased tissue suggests that the role of this enzyme is slight in pathogenesis of the scab organism on cucumber. High Cx activity was demonstrated from similar samples. In addition, symptoms of the disease are characteristically local with extensive tissue disintegration. It is therefore concluded that Cx probably plays an important role in the disintegration of cucumber tissue by *C. cucumerinum*.

The commercial cellulase preparation used in these studies was extremely thermostable, whereas cellulase-active filtrates of *C. cucumerinum* were rendered inactive by boiling. These results suggest that at least 2 different cellulolytic enzymes were involved, unless a very efficient buffer system was associated with the thermostable preparation. Variability in heat stability of cellulase-active preparations has been reported by many workers (6, 8, 10, 12, 17). Such variation in thermostability of substances which reduce viscosity of CMC tends to support the multi-enzymatic cellulase theory of Reese (11).

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SOME METABOLIC CHANGES ACCOMPANYING INFECTION BY BARLEY YELLOW DWARF VIRUS

G. B. Orlob and D. C. Arny

Department of Plant Pathology, University of Wisconsin, Madison.

Present address of senior author: Department of Plant Pathology, South Dakota State College, Brookings.

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SUMMARY

Relative data were collected on the physiology of barley plants infected with yellow dwarf virus by comparing healthy and diseased plants of the same variety, age, and cultural conditions. Respiration rates were higher than normal during early stages of infection, but lower from 35-69 days after inoculation. Photosynthesis, as well as transpiration, decreased in infected plants. In infected leaves, starch and soluble carbohydrates accumulated, especially reducing sugars. Soluble carbohydrate content of infected roots was lower than that of healthy roots. Virus infection affected the nitrogen metabolism by reducing total-N in leaves and increasing it in the roots. Infected leaves contained less protein-N. In addition to metabolic changes, some alterations of an enzymatic nature were noticed: catalase activity decreased in infected leaves, and peroxidase activity was at a higher level. Metabolic disturbances in a tolerant barley variety were in the same direction but less pronounced than in a susceptible variety.

Introduction.—Virus infection usually results in drastic changes of biochemical and physiological processes of the host (1). In spite of numerous investigations, pathologically altered metabolism is poorly understood. No conclusion as to the underlying mechanism of virus-host interaction has yet emerged. Nevertheless, the metabolic reactions of the host might be used in setting up characteristics of a disease as a supplement to symptomology and histopathological changes. According to macroscopic symptoms, barley

yellow dwarf (BYD) can be classified as a typical aphid-borne, yellows-type disease. Diseases of this type are characterized by certain metabolic changes which appear closely linked to morphological-anatomical disturbances, mainly in the vascular tissue of the host (5, 38). The purpose of the present study was to collect some preliminary data on the altered metabolism of susceptible and resistant small grain varieties and to decide whether the effect of BYD on its host is typical of a yellows-type virus disease.

RESPIRATION.—As early as 1912, the increase of respiration of leaf roll-infected potato plants was noted. Subsequently, many workers found that respiration increased after infection with various viruses (1, 2, 37). Some investigators, however, reported a lower rate of respiration in virus-infected plants or that the respiration pattern was affected differently by different viruses (4). Owen (22), in his work with tobacco mosaic virus (TMV), concluded that the respiration rate of virus-infected leaves could be lower or higher than, or identical with, that of healthy leaves depending on time after inoculation, environmental conditions, leaves used for assay, or mode of expression of results. His account may explain contradictory results obtained in earlier work. Increased respiration, however, occurs also during normal plant life. Characteristic of the course of respiration in aging leaves is a phenomenon known as "climacteric rise." Shortly before chlorosis appears in leaves, respiration increases and then decreases after reaching a climax. One of the physiological results of virus infection is a hastening of the process of aging (2). Such aging effects alone could be responsible for metabolic changes in infected plants.

In the present work, determination of respiration rates was made on 2 barley varieties [C.I. 666 (susceptible) and C.I. 2376 (resistant)] grown at 20°C and inoculated in the 2-3-leaf stage by means of *Macrosiphum* (*Metopolophium*) *dirhodum* (Wlk.) (rose grass aphid). O_2 uptake of healthy and infected leaves (2 youngest) was measured at several intervals 5-69 days after infection using the Warburg method as described by Umbreit, *et al.* (33). In each experiment, 3 reaction flasks of infected and 3 of healthy leaves were used. Measurements were carried out at least twice. Experiments were made in the dark.

Respiration rates of the 2 barley varieties are shown in Fig. 1. The rate was higher in infected leaves shortly before symptoms appeared (Fig. 1-A, B) and continued at an increased level up to 20 days (Fig. 1-C). This tendency was found in both the susceptible and resistant varieties, although the latter seemed less stimulated. Respiration rates of infected leaves were lower than those of healthy leaves 37 days after infection, and in all subsequent tests (Fig. 1-D).

When apparently healthy and infected leaves of a susceptible oat variety (C.I. 7107) were collected in the field and respiration measured as described above, infected leaves showed augmented respiration comparable to that of barley (C.I. 2376) (Fig. 1-C). Apparently, the plants had been infected with BYDV for 15-25 days.

The respiration pattern of infected plants apparently may vary with the virus involved. Owen (21) reported that respiration rates began to change less than 1 hour after inoculation with TMV, whereas he later (23) found that a strain of tobacco etch virus (TEV) did not change respiration rates of infected leaves until external symptoms developed. Barley leaves infected with BYDV showed augmented respiration 5 days after inoculation. Symptoms usually appeared in 8-12 days. A drop in respiration during the later stages of infection has been found in other virus diseases (38). In the case of plants infected with TEV, however, increased respiration occurred at all times of the year and was maintained throughout the life of the leaves (23).

PHOTOSYNTHESIS.—One of the commonest symptoms of virus diseases is a chlorosis of the infected leaves. Analyses made by several investigators have indicated that the chlorophyll content of virus-infected plants was reduced either by a retarded synthesis of chloroplasts or by their destruction. This reduction of photosynthetic centers is followed by a reduction in photosynthesis both in mosaic-type (24) and yellows-type diseases (20, 26).

For measurement of photosynthesis, comparable leaves of the 2 barley varieties were selected 22 days after infection with BYDV; in addition, photosynthesis of the resistant variety was determined 40 days after infection. The method used was the "one-vessel" procedure described by Umbreit, *et al.* (33). In addition to the normal fluorescent light in the laboratory, two 200-w incandescent bulbs were attached to the Warburg apparatus approximately 10 in. above the reaction vessels. The rate of photosynthesis (h-Photo) was calculated from the "steady state of gas exchange method" (33). Experiments were run 3 times.

The average rate of photosynthesis of the susceptible variety 22 days after infection was decreased when compared with that of the healthy leaves as follows:

$$\text{Infected h-Photo} = 0.225 \text{ mm}$$
$$\text{Noninfected h-Photo} = 0.397 \text{ mm}$$

In the resistant variety, no changes of photosynthesis could be observed either at the 22- or the 40-day interval. Although the failure to detect changes of photosynthesis in this variety could have been due to the low sensitivity of the method used, photosynthesis in the resistant variety was much less affected than in the susceptible variety.

These experiments indicate that damage to the photosynthetic mechanism parallels virus-induced damage of the chloroplasts expressed in the amount of leaf yellowing. Spikes and Stout (30) found reduced chloroplastic activity in sugar beets infected with yellows virus. They concluded that the virus could decrease photosynthetic sugar production in the plant by a direct action on the chloroplasts, rather than by some indirect action on the photosynthetic mechanism. Other investigators have postulated dif-

ferent mechanisms, *e.g.*, accumulation of carbohydrates, as a possible cause of reduced photosynthesis. Müller (20), however, demonstrated that photosynthesis of leaf roll-infected potato leaves was reduced even when no accumulation of starch occurred. Whatever the influence of the virus on the photosynthesis might be, a drop in photosynthesis, together with an increase in respiration, constitutes a serious

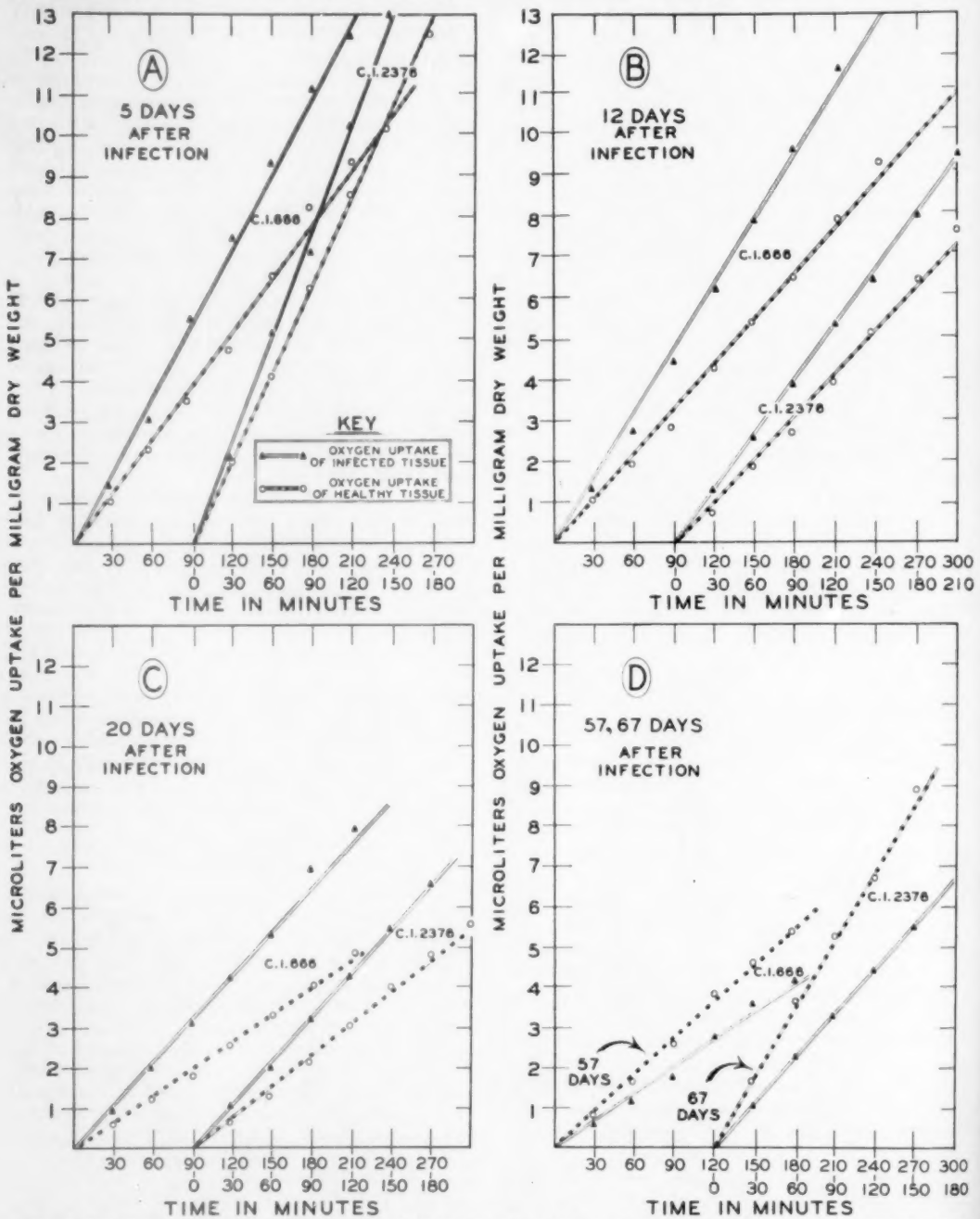

Fig. 1. Relative respiration rates in leaves from healthy and infected plants of resistant (C.I. 2376) and susceptible (C.I. 666) barley varieties at various intervals after infection. Graphs are from single experiments but are representative of several test runs.

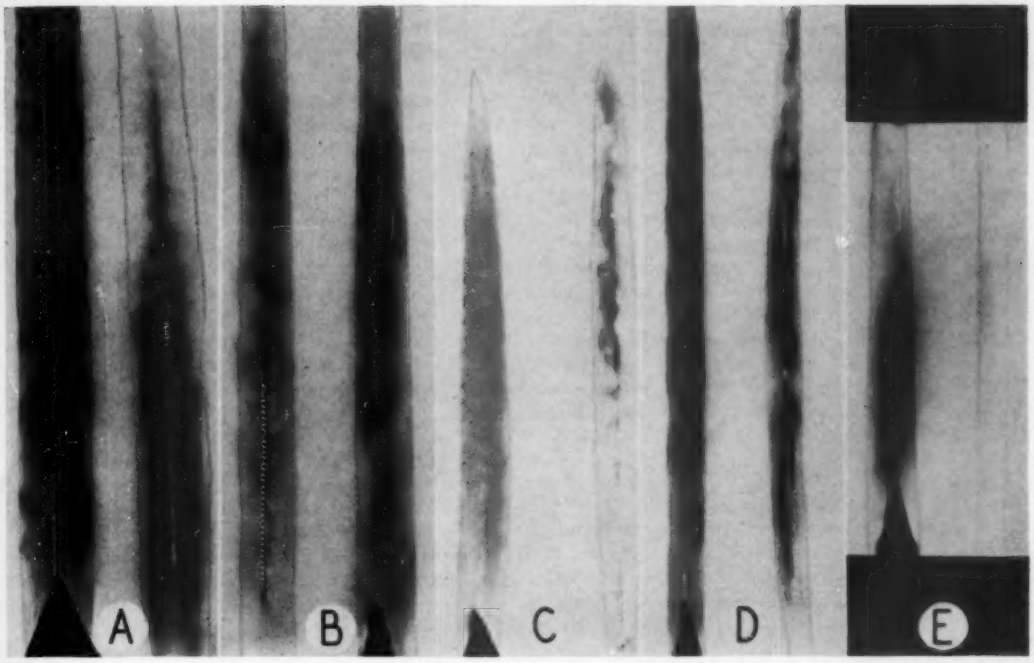

Fig. 2. Transpiration of leaves from healthy and virus-infected plants of C.I. 666 (susceptible) and C.I. 2376 (resistant) barley varieties. Healthy leaf (notched) on left except in **B**). **A)** C.I. 666 at 24 days after inoculation. **B)** C.I. 2376 at 24 days. **C)** C.I. 666 at 11 days after inoculation. **D)** C.I. 2376 at 11 days. **E)** C.I. 666 at 20 days, leaves placed in water for 12 hours prior to measurement.

condition for the plant, and may in turn be responsible for alterations of other functional relations.

TRANSPIRATION.—Pantanelli (25), in 1912, studied the effect of virus infection on the rate of transpiration of grape leaves and stated that normal tissue transpired about 8 times as much as did diseased plants. Gondo (9), working with TMV, found no great difference between transpiration rates of healthy and diseased tobacco plants for 1 month after inoculation, although thereafter the rate in diseased plants was significantly lower than that of healthy ones. Schuster (27), however, reported increases in transpiration after the infection with several mosaic-type viruses paralleled by an increase of symptom severity.

Transpiration in the 2 barley varieties was studied by the hygrophotography method described by Sidvadjian and Kern (28), which is rather simple and relates results to the leaf surface. Photographic plates are treated so that the emulsion will change its color and translucency on contact with water vapor. Leaves assayed were detached from the plant and immediately transferred to the photographic plates, covered with a glass plate, and left there until a yellow silhouette appeared in areas where the leaf transpired. After exposure, the plates were kept dark and dry and were printed on photographic paper as soon as possible.

Shortly before the first sign of macroscopic symptoms appeared (approximately 8-12 days after infection), transpiration rates began to decrease (Fig. 2). During the later stages of the disease, transpiration of the infected leaves tended to be lower than those of the healthy ones. In the susceptible variety, transpiration was especially decreased in areas where yellowing had occurred. Transpiration of the resistant variety was less affected by virus infection than that of the susceptible variety (Fig. 2).

To eliminate possible effects of reduced absorption by roots, excised leaves were placed in distilled water for 12 hours. Estimation of the transpiration after this period still indicated lower transpiration from infected leaves (Fig. 2-E), although the rate of water loss of the healthy leaves also was reduced greatly.

A decreased transpiration could be explained in several ways. While the direct effect of the virus is on the phloem, accumulation of gums in the xylem and reduction in xylem development have been noted (6). The roots of yellow dwarf-infected plants are severely reduced and eventually the water absorption decreases. The reduced ability of the plant to absorb water could be balanced by a reduced rate of transpiration. Similarly, in the present study, water content of small-grain plants infected with BYDV also was decreased. A decrease in the total volume of water in a plant could be compensated by a reduction in transpiration. Müller (20) reported that stomata of potato leaves infected with leaf roll virus were more closed than in healthy leaves. Diminution in stomatal

transpiration would explain the decreased loss of water vapor. This was not checked in BYDV-infected plants.

CARBOHYDRATE METABOLISM.—Virus diseases may be divided into 2 classes according to the effects of the disease on the carbohydrate metabolism (38). Mosaic diseases often are accompanied by a decrease in total carbohydrates; whereas, in yellows-type diseases, carbohydrates accumulate. Early research showed a high percentage of carbohydrates, starch as well as sugar, in leaf roll-infected potato leaves (38). More recently, reducing sugars were found by Martin (15) 6-10 times as high in leaves affected by leaf roll virus as in healthy ones, glucose having increased most. Similar results were obtained in experiments dealing with beet yellows as reported by Henke (10). Total sugars in the roots were reduced by 30-40%, but were increased by 33% in the leaves. Sommer (29), Stange (31), Steudel and Heiling (32), and Watson and Watson (36), all working with beet yellows, obtained similar results.

Starch.—Starch accumulation was estimated by immersing sections of leaves in 95% ethyl alcohol for 12-14 hours to remove chlorophyll and then placing the sections in an IKI solution.

These experiments showed that infected leaves had a higher starch content than healthy leaves, as Watson and Mulligan (35) had previously found. Differences were less marked in the resistant varieties. Accumulation occurred shortly before the onset of symptoms and continued throughout the later stages of the disease. The amount and distribution of starch fluctuated greatly during the day. Differences between healthy and diseased leaves were most striking when samples were taken during the late afternoon or evening hours; there was no difference in morning samples.

Soluble carbohydrates.—The anthrone method of Morris (19) was used to compare the soluble carbohydrate content of virus-infected tissue with that of healthy tissue. One-4 g of fresh plant material were weighed and finely ground in 100 parts of water. The macerate was allowed to stand for 0.5 hour, 3 ml of the supernatant were measured into a test tube, and 6 ml of the anthrone reagent were added. After a heat treatment and the development of a blue color, the extract of a virus-infected tissue was compared with 1 of healthy tissue by means of a Klett-Summerson colorimeter (with the extract of lower carbohydrate content set at 0). This gave a relative measure of the difference, as no standards were used.

The comparisons revealed an increase of soluble carbohydrates in virus-infected leaves of C.I. 666 (Table 1). This difference was accentuated with more time after inoculation. Differences were less pronounced in the resistant C.I. 2376. When oven-dried leaves were used instead of fresh ones, the differences were more pronounced. Roots tested 37 days after infection showed a decrease in soluble carbohydrate content (Table 1). Accumulation of carbohydrates in infected leaves could be overcome by placing diseased plants in the dark for 3-4 hours. Studies on a resistant and a susceptible variety and F_3 lines of crosses between these 2 varieties revealed that the extent of carbohydrate accumulation was generally correlated with macroscopic symptoms.

Reducing sugars.—For estimation of reducing sugars, paper chromatography was used, since it allowed a differentiation between sugars and other reducing materials in the extract. Plant extracts were prepared as described by Stange (31). The chromatogram was developed on Whatman No. 1 paper in the ascending manner with ethyl acetate:acetic acid:water as the developing mixture (3). The relative amounts of materials in the various tissues were determined by estimating size and color intensity of the spots. Solutions of glucose and fructose were run as controls.

Reducing sugars also increased in infected leaves. This increase was greater in the later stages of the disease and greater in the susceptible than in the resistant variety. Largest differences in the amount of reducing sugars were noted between infected and apparently healthy leaves of field-grown oats (C.I. 7107), collected during the last days of June approximately 15-20 days after natural infection with BYDV.

The above results indicated that changes in the carbohydrate metabolism of BYDV-infected plants compare with those in other yellows-type diseases. Prevention of translocation by degenerative changes in the phloem has been suggested as the possible cause of an accumulation of carbohydrates. Phloem necrosis was shown by Esau (5) in plants infected with BYDV. In beet yellows, Watson and Watson (36) and Stange (31) concluded, however, that accumulation of carbohydrates could not be attributed to a reduced translocation, since loss of starch and total carbohydrates was as great from leaves during a dark period as from healthy leaves. In the present study, there was no accumulation of starch following a period of low light intensity during the night. Accumulation of soluble carbohydrates in infected leaves was reduced considerably when these plants were kept in the dark. Bawden (1) discussed the possibility that a rise in carbohydrate content of the leaves, without any change in the rate of translocation, might result from an increased resistance to the movement of sugar out of the leaves, so that a higher concentration had to be built up before outward movement took place.

NITROGEN METABOLISM.—Reports in the literature indicate that infection with certain viruses increases the N level of the leaves, while others reduce it: an increase is characteristic of mosaic-type diseases; a decrease, of the yellows-type disease. Results, however, have been somewhat inconsistent. As reported by Henke (11), potato leaf roll caused decreases in total-N and protein-N, with quantitative differences between varieties. Steudel and Heiling (32), working with beet yellows, stated that the N metabolism in the tops was characterized by increased proteolytic processes followed by a reduction in insoluble-N and total-N contents, whereas the N-content of diseased roots was greatly increased. Similar results were obtained by Watson and Watson (37).

TABLE 1. Summary of biochemical analyses of yellow dwarf virus-infected barley plants in comparison with healthy plants

Constituent analyzed and variety used	Relative level of constituents at given no. of days after virus infection															
	7 days				17 days				27 days				37 days			
	1[a]	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.
LEAVES																
Soluble[b] carbohydr.																
C.I.666	31	34		*32*	90	100	86	*92*	100	130		*115*	130	144	170	*148*
C.I.2376	30	36		*33*	55	45	41	*47*	37	41		*39*	50	36		*43*
Total-N[c]																
C.I.666	83	85	80	*83*	78	83	80	*80*	77	78	82	*77*				
C.I.2376	94	103	95	*97*	94	93	90	*92*	96	94	97	*96*				
Protein-N[c]																
C.I.666									75	78	83	*79*				
Soluble-N[c]																
C.I.666									95	103		*99*				
Catalase[c]																
C.I.666	67	64	71	*67*	60	64		*62*	67	70		*68*				
C.I.2376	75	79	76	*77*	77	76		*77*	90	82		*86*				
Peroxidase[c]																
C.I.666	148	149		*149*	127	131		*129*	128	130		*129*				
C.I.2376	114	115		*115*	117	112		*114*	112	106		*109*				
ROOTS																
Soluble carbohydr.																
C.I.666													160	120	190	*157*[d]
Total-N[c]																
C.I.666													158	169	191	*173*
Catalase[c]																
C.I.666													151	142		*147*

[a]Number of experimental series, Avg. represents average from all experiments.
[b]Dial reading on colorimeter, healthy control set at 0.
[c]Index in percentage of healthy (= 100).
[d]Dial reading on colorimeter, infected tissue set at 0.

In the present study, N determinations were made in triplicate by the semimicro-Kjeldahl method. The measurements were calculated as mg/g of dry plant tissue, but the results are in terms of infected plants as percentages of the healthy controls (Table 1). In the estimation of protein-N, leaves were finely ground in distilled water, crude cell material was centrifuged off, and protein was precipitated by 2% trichloroacetic acid or by heating. Comparisons then were made visually or by Kjeldahl determinations on the precipitate.

The total-N of infected leaves of the susceptible C.I. 666 variety decreased during the first week after inoculation and was at a low level thereafter (Table 1). As with other constituents, differences between infected and healthy leaves were less obvious in the resistant variety C.I. 2376. When leaves of susceptible plants were analyzed 27 days after infection, the decrease in total-N apparently was due to the decrease in protein-N since the soluble-N was not changed materially. This decrease in protein-N could be visualized in field-collected leaves of a susceptible oat variety (C.I. 7107), when proteins were precipitated by heating. There was much less flocculant in the infected leaf extracts than in the control. Differences in protein-N were less obvious in greenhouse-grown barley leaves. The total-N content of roots of infected barley (C.I. 666) was higher than that of healthy ones (Table 1).

From the data obtained, it can be concluded that, in BYDV-infected plants, changes in the N metabolism are typical for a yellows-type disease. Wynd (38) discussed causes of variations in the amount of N, such as disturbed translocation and the dilution effect of accumulating carbohydrates. Diminishing N content after infection also could be related to an inhibition of protein synthesis. It is generally believed that the ability to form protein is maintained as long as chloroplasts are present, and it has been demonstrated that the protein content of the green part was much higher than the yellow part of variegated leaves of a number of plants (17). BYDV causes a severe yellowing in

susceptible barley varieties. Low content of total-N and protein-N could be caused by a destruction of the photosynthetic centers, comparable to the conditions in variegated leaves. Evidence for an inhibited protein synthesis was obtained by Henke's (11) work in which leaf roll-infected potato plants recovered after the application of egg protein.

CATALASE AND PEROXIDASE ACTIVITY.—Several authors (16, 34) have demonstrated an increase in peroxidase activity of virus-infected tissue. Farkas and Király (7) have stated that an increase in enzyme activity was in agreement with the idea that strong respiratory activity of the host tissue favors virus development. On the other hand, some enzyme systems seem depressed (29). Although opposite results were sometimes reported in early work (38), several authors recently have found low catalase activity in virus-infected plants (7, 14, 34). Other investigators, however, have found that enzymatic activity is changed during certain normal or abnormal, but not necessarily infectious, conditions of the plant; *e.g.*, catalase activity was lower in chlorotic leaves (18), whereas dwarfed plants had a higher peroxidase activity (13). Both yellowing and dwarfing are typical symptoms of BYD.

In the present study, small amounts of tissue of C.I. 666 and C.I. 2376 were weighed, finely macerated in a mortar, and diluted with phosphate buffer in ratios of 1:10-1:100 depending on enzyme concentration. The supernatant was used for estimation of catalase (8) and peroxidase activity (12). Boiled plant homogenates served as checks. Measurements were made in seconds of reaction time, but expressed in terms of infected as percentages of noninfected tissues (Table 1). The figures in Table 1 represent an average of 40-60 single determinations for both infected and noninfected tissues.

Virus infection affected the activity of the 2 enzyme systems differently (Table 1). Peroxidase activity was higher and catalase activity lower in infected leaves than in healthy leaves. These differences were well established during the first week after infection and prevailed for at least 3 more weeks. Values for the resistant variety (C.I. 2376) showed the same tendency as those for the susceptible one, but differences were less marked. In contrast to leaf material, catalase activity in infected roots was higher than in healthy roots when tested 37 days after infection.

Data obtained in the present studies generally confirmed the results obtained by Vager (34) who reported that virus infection of tobacco and tomato leaves stimulated peroxidase activity, whereas the activity of catalase decreased. Similarly, Martin (16) recorded an increase of peroxidase activity in tobacco leaves infected with TMV and a combination of TMV and potato virus X.

DISCUSSION.—A disease manifests itself by both general and specific symptoms. These 2 aspects combine to form the diagnostic picture of the disease. Gross changes in the rates of respiration and photosynthesis, for example, must be considered general effects of a disease. Disturbances of the carbohydrate and N metabolism, as well as their respective enzyme systems, resulting from altered rates of assimilation or in abnormal distribution in the host tissues, are believed more specific for a given virus-host combination. From this point of view, it has been shown that metabolic changes in plants infected with BYDV are quite similar to those reported from other yellows-type diseases.

The question may arise: how specific are these "virus-specific" reactions? Virus infection certainly causes typical changes in the host metabolism that might be used as a basis of chemical virus diagnosis. In various sections, it has been pointed out, however, that comparable disturbances occur not only in infected plants, but also can appear during certain stages of normal plant life (*e.g.*, aging), under abnormal conditions (*e.g.*, chlorosis or dwarfing), or can be induced by environmental factors. In this connection, it should be remembered that macroscopic symptoms of BYDV or histopathogenic changes in infected tissue are not entirely specific for the disease but can be induced by a variety of conditions or substances (6,. On the other hand, at least the protein metabolism is specifically changed following virus infection: this problem has become a central part of modern virus research. Thus, more extensive and detailed biochemical studies are needed to define clearly the influence of virus infection on the host metabolism and to determine to what extent different viruses affect host metabolism differently.

Recently, more attention has been focused on the comparative physiology of disease-resistant and susceptible plants. In some instances, resistance to virus infection is governed by hypersensitivity leading to the exclusion of the virus by antagonistic reactions and subsequent death of the invaded cells. In the case of BYDV, both the resistant and susceptible plants were infected and invaded by the virus. Whereas the susceptible variety developed severe macroscopic symptoms and metabolic disturbances, the resistant variety did not react in a perceptible way to virus infection and developed less pronounced metabolic changes, *i.e.*, it tolerated the virus. Tolerance to BYDV appeared relatively rare among varieties of oats and barley, but a number of grasses are symptomless carriers of the virus.

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FREE AMINO ACID CONSTITUTIONS OF HEALTHY AND SCAB-INFECTED CUCUMBER FOLIAGE

Clyde L. Burton and Donald J. deZeeuw

Department of Botany and Plant Pathology, Michigan State University, East Lansing.

Respectively, former graduate student, Department of Botany and Plant Pathology, now Plant Pathologist, Niagara Chemical Div.; and Associate Professor, Department of Botany and Plant Pathology, Michigan State University.

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SUMMARY

Sixteen ninhydrin-positive amino acids and amides were identified in aqueous extracts of both scab-resistant and scab-susceptible cucumber leaf tissue. Aspartic acid, serine, glutamic acid, glycine, asparagine, lysine, glutamine, threonine, histidine (or arginine), citrulline, α-alanine, γ-aminobutyric acid, α-aminobutyric acid, pipecolic acid, valine, and leucine(s) were consistently isolated on paper chromatograms. Pipecolic acid is relatively uncommon in plants, but conspicuous in cucumber. Asparagine and glutamine were markedly reduced in severely diseased tissues. This reduction became apparent 72 hours after inoculation and coincided with the first symptoms of infection. The amides had completely disappeared after 120 hours when tissue destruction was extensive.

Scab of cucumber, caused by the fungus *Cladosporium cucumerinum* Ell. & Arth., has been used in various studies on parasitism. Neither an anatomical (10) nor a genetic approach (11) has solved some of the questions on metabolic changes during the infection process.

C. cucumerinum, a facultative parasite which exists on a variety of nutrients and produces clear-cut disease symptoms on cucumber foliage, was chosen for a series of studies. Resistance to scab infection in the seedling stage is correlated with resistance in the fruit of mature plants (11) and is a simple dominant characteristic. Sister varieties of cucumber, approaching the isogenic condition for scab resistance, also were available. Presumably, such closely related lines would best reflect metabolic changes due solely to infection by the parasite.

Paper chromatography has been used extensively in recent years in analyzing the amino acid constitution of plants. The amino acids are believed important in plant resistance mechanisms, and various changes have been shown in diseased plants. Resistance to soybean stem canker may increase with an increase in amino acid content (1); a virus infection may lead to increases in certain water-soluble or free amino acids (8); and losses of certain amino acids may occur following rust infection (5). Resistance to apple scab was increased by infiltrating D- and DL-phenylalanine into apple shoots (7).

Since no general pattern of amino acid change in parasitism has been demonstrated, further evidence should prove useful.

MATERIALS AND METHODS.—Four pickling cucumber varieties were used. Yorkstate is an open-pollinated, scab-susceptible, mosaic-resistant variety. Maine No. 2 is an open-pollinated, scab-resistant, mosaic-susceptible variety. The inbred sister lines CS-2W and CS-2M were respectively scab-susceptible and scab-resistant. Both CS-varieties were mosaic-resistant selections from repeated back-crosses of Yorkstate on Maine No. 2 (Michigan Agricultural Experiment Station Project 156).

Inoculations were made with a heavy spore suspension of several pathogenic scab isolates grown on potato-dextrose agar. Incubation was in a controlled-temperature moist chamber held at 17°C.

Sample experimental leaves were taken at appropriate times in the experiments; some were used for respiratory analysis (4). The remaining leaf samples were prepared for paper chromatography by a modification of the techniques of Block, *et al.* (2) and Hrushovetz (6). The tissue was frozen, homogenized with ethanol, dried at 60°C, and ground. For spotting, the dried material was extracted in ethanol. Water-soluble fractions were separated from fats by chloroform extraction.

Portions of the undiluted extract were applied uniformly to small circular areas marked a standard distance from the edge of the paper (Whatman No. 1, 18 x 22 in.). All suitable precautions were taken to apply known and equivalent quantities of the various extracts (3). The mobile phases for the 2-dimensional descending chromatograms were N-butanol-acetic acid-water (25:6:25 by volume) and phenol-triple distilled water (4:1 w/v). Standards made from known combinations of pure amino acids (California Corporation for Biochemical Research) were included with each run; all reasonable controls were applied to time, temperature of run, and development. The chromatograms were developed by spraying with a 0.25% v/v solution of ninhydrin in acetone. The color areas were outlined with pencil and identified at 24 hours. The different samples were compared and the color density noted. Ultraviolet light examination of unstained chromatograms was found unsuitable for the materials analyzed.

RESULTS.—Plants of the 4 varieties were grown to the beginning of the 2-leaf stage. Half were inoculated

and incubated; the remainder were incubated without inoculum. Samples were taken at mid-morning each day for 5 days to control diurnal effects. The part reserved for chromatography was preserved and analyzed after respiration had been determined for all stages of infection.

The varieties CS-2M and CS-2W, considered the most important because of their genetic similarity, were studied in most detail. Because of experimental difficulties, only the samples at 24, 72, and 120 hours were replicated enough times to be highly reliable.

At the 24-hour sampling, 16 ninhydrin-positive spots were identified as typical of all varieties and treatments — aspartic acid, serine, glutamic acid, glycine, asparagine, lysine, glutamine, threonine, histidine or arginine, citrulline, α-alanine, γ-aminobutyric acid, α-aminobutyric acid, pipecolic acid, valine, leucine(s). One of these and a seventeenth spot were considered a separation of glutamic acid. The leucines, not separated by the method used, were considered a unit. The amino acid-amide array of the uninoculated plants of all varieties remained essentially unchanged throughout the experiment.

At 24 hours, the 4 inoculated varieties were the same as the controls in amino acid-amide constitution. At 72 hours, however, the inoculated susceptible varieties showed less glutamine and asparagine than did the resistant uninoculated plants. Chromatograms from inoculated susceptible material at 120 hours had no identifiable ninhydrin reaction for either glutamine or asparagine. The other amino acids appeared unchanged in concentration in all cases, regardless of variety, inoculation, or stage of incubation.

A preliminary chromatographic study was made on the *in vitro* utilization of amino acids from cucumber leaves. Good growth of the organism was obtained on leaf extracts of both resistant (CS-2M) and susceptible (CS-2W) varieties. Most of the ninhydrin-active compounds appeared absent after 3 weeks of growth and the others reduced substantially compared with the controls. In this case, no selectivity was evident for glutamine or asparagine.

DISCUSSION.—The disappearance or reduction of net asparagine and glutamine in infected susceptible cucumber leaves was coincident with initiation of visible symptoms and certain respiratory changes (4). This relationship must be of significance in parasitism not only because of the importance of these 2 nitrogenous compounds in the plant's metabolism generally, but also because it is doubtful that asparagine and glutamine are used selectively as nutrients by the parasite. Neither the behavior of the fungus *in vitro* nor chromatographic analysis of affected plants tends to support such a static mechanism. No other change could be found in the ninhydrin-active compounds of diseased versus healthy tissue.

Glutamine and asparagine play an important part in nitrogen metabolism of plants by preventing the accumulation of free ammonia (9). They appear in plants when large quantities of ammonia ions are being absorbed by the roots and when reserve proteins in young seedlings are being digested to supply energy for growth. They also are evident in instances of carbohydrate starvation, when amino acids and amides are oxidized during respiration, setting free large quantities of ammonia. The free ammonia is utilized in amide synthesis. This suggests that disappearance of glutamine and asparagine results from any action by the pathogen preventing movement or availability of ammonium ions in the leaf. Translocation pathways between leaf blade and stem are, in fact, blocked effectively by early necrosis of much of the vascular tissues and parenchyma in the petioles.

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PATHOLOGICAL HISTOLOGY, HOSTS, AND CULTURE OF THE POTATO ROT NEMATODE

L. R. Faulkner and H. M. Darling

Department of Plant Pathology, University of Wisconsin, Madison.

Respectively, former Graduate Assistant in Plant Pathology, University of Wisconsin, now Assistant Nematologist, Washington State University, Irrigation Experiment Station, Prosser; and Professor of Plant Pathology, University of Wisconsin.

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SUMMARY

Aseptic cultures of fungi and undifferentiated callus tissues of carrot, potato, clover, and tobacco were used as hosts for increasing the potato rot nematode. By isolating 190 single gravid females of *Ditylenchus destructor* Thorne on petri-plate fungus cultures, 16 pure colonies of this nematode were obtained. The isolates did not vary in morphology or lose pathogenicity to potato after 4 years of continuous culture.

Cultures of 115 species of fungi, the majority of which were common soil forms, were inoculated with uncontaminated nemas from a pure colony. *D. destructor* fed and reproduced on 64 species of fungi, representing 40 genera, 8 orders, and all major classes.

When potatoes were inoculated with a host fungus and the nematode, the severity of disease was markedly increased.

Carrot, clover, alfalfa, radish, sweet potato, parsnip, and bulbous iris were suitable hosts for the nematode. Alfalfa, parsnip, and radish have not been reported as hosts previously.

A study of the pathological histology of diseased potatoes indicated a progression in the development of tuber rot. Apparently, the nematode is a primary parasite capable of causing considerable damage to potato in the absence of other organisms. Secondary invaders, however, primarily fungi, were necessary for the advanced stages of the disease.

INTRODUCTION.—The first recognition of a tuber rot incited by a nematode was by Kühn in 1888 (13), who identified the causal organism as *Anguillula dipsaci* Kühn and later changed the name to *Tylenchus devastatrix*. In 1936, Filipjev renamed it *Ditylenchus dipsaci* (Kühn) Filipjev. In 1943, Blodgett (3) observed a potato malady in a field near Aberdeen, Idaho; and tentatively identified the associated nematode as *D. dipsaci*. In 1945, Thorne (15) compared nematodes from Idaho with known specimens of *D. dipsaci* and found differences on which he based a new species (*Ditylenchus destructor* Thorne), now known as the potato rot nematode. In 1945, Baker (1) found this organism attacking potatoes on Prince Edward Island, Canada. The nematode was found near Antigo, Wis., in 1953 (6). That same year, Bosher (4) reported that the nematode of bulbous iris (later identified as *D. destructor*) caused potato rot in British Columbia. Currently, this species has been reported only from the previously mentioned locations in North America, but is quite common throughout Europe.

Reported hosts for the potato rot nematode are: potato (*Solanum tuberosum* L.), carrot (*Daucus carota* L.), butter and eggs (*Linaria vulgaris* Hill), mint (*Mentha arvensis* L.), common blue-eyed grass (*Sisyrinchium angustifolium* Miller), grassleaf goldenrod (*Solidago graminifolia* (L.) Salisb.), field sowthistle (*Sonchus arvensis* L.), common dandelion (*Taraxacum officinale* Weber), alsike clover (*Trifolium hybridum* L.), red clover (*T. pratense* L.), common vetch (*Vicia sativa* L.), rippleseed plantain (*Plantago major* L.), sugar beet (*Beta vulgaris* L.), common hop (*Humulus lupulus* L.), common lilac (*Syringa vulgaris* L.), tigerflower (*Tigridia pavonia* Ker.-Gawl.), wreath nasturtium (*Tropaeolum polyphyllum* Cav.), woundwort (*Stachys palustris* L.), and iris (*Iris xiphium* Dutch.) (4, 5, 8, 9, 10, 13). Henderson (10) and Goodey (9) indicated that the host species had a marked influence on the dimensions of *D. destructor*. These workers inoculated plants with nemas which had been removed *en masse* from naturally infected tubers, or used infested soil as the source of nemas; therefore, they could not be certain their inoculum was pure. Techniques have since been developed which make it possible to inoculate plants with pure populations of *D. destructor* under controlled conditions (7). Krusberg (12) recently reared *D. dipsaci* and *Aphelenchoides ritzemabosi* on alfalfa callus in culture.

The presence of *D. destructor* is difficult to determine under field conditions because the only symptoms are on the tubers. Larvae enter the developing tuber when it is small, approximately 1.0-1.5 in. in diameter. By removing a thin peeling from these tubers, small chalky spots are usually found, characteristic of early infections. Examination of chalky areas under the dissecting microscope reveals the presence of 1 to many nematodes. By harvest time, secondary organisms have invaded the tissues and tuber breakdown is conspicuous. Diseased tubers are characterized by skin checking, cracking, and rotting similar to certain dry rots in stored potatoes (Fig.1).

Examinations of infected tubers revealed that the disease did not progress in storage beyond the "chalky area" stage unless secondary organisms had subsequently invaded the tuber. Large numbers of nemas were usually not found in infected areas unless fungi

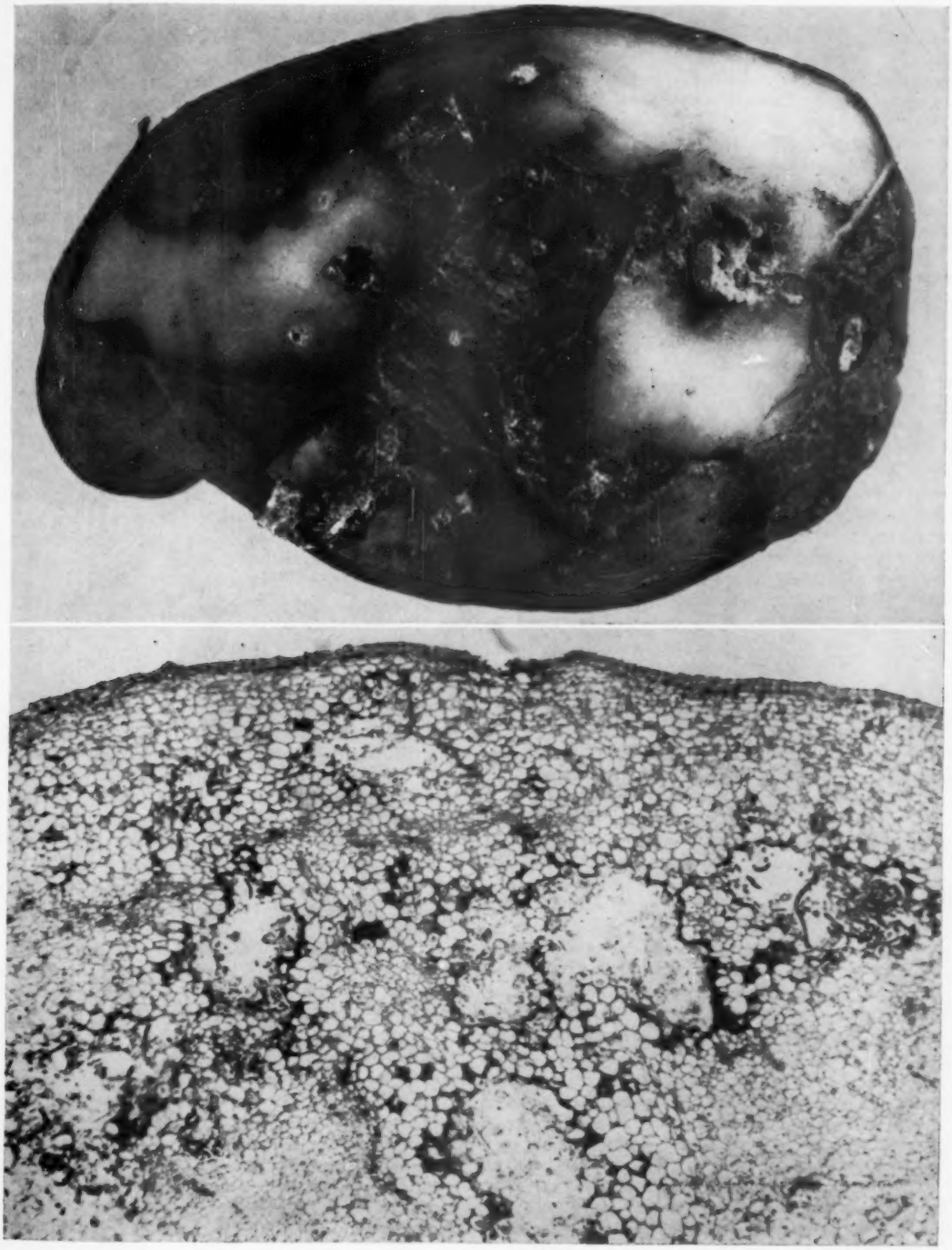

Fig. 1. (Above) Potato tuber infected with the potato rot nematode. Note the small white chalky areas which are characteristic of the disease. (Below) Section of lesion from a potato tuber showing reticulate system of tunnels and cavities.

were present. The role of fungi in potato rot, however, has not been clearly determined. Some workers have felt that the presence of fungi in rotted areas is purely coincidental since *D. destructor* was considered the primary parasite. Other workers believed that fungi played an important role in the disease, either by serving as survival hosts for the nematode between potato crops or by producing additive or synergistic effects in tuber rot. Baker, *et al.* (2) suggested a definite relationship between the potato rot nematode and secondary organisms in disease development. These workers stated that nemas would evacuate the infected tubers when bacterial wet rots progressed rapidly. They also suggested a close relationship between the nematode and fungi, since *D. destructor* can be reared on fungi in culture. Three *Fusarium* spp., a *Mucor* sp., and *Trichoderma viride* (Pers.) Fr. were listed as fungus hosts for the nematode.

The present investigation is concerned with the role of fungi in the development of tuber rot, the histology of the disease, the hosts of the incitant, and behavior of this nematode on tissue cultures and on isolates of several fungi.

Materials and Methods.—Aseptic cultures of fungi and *in vitro* plant tissues were employed in rearing nemas. Fungi were cultured on clarified acidified potato-dextrose agar in petri dishes. The medium for plant tissue cultures was a modification of White's nutrient agar medium (17) and was contained in 6-oz. medicine bottles.

Sterile petri dishes, containing acidified potato-dextrose agar, were inoculated with a selected fungus. After 4-5 days of incubation, gravid females were transferred through 3 baths of sterile distilled water and placed approximately 1.5 in. from the fungus colony. Movement of the nemas through this agar apparently removed all bacterial contaminants from the body surface; for, after they reached the fungus, little or no bacterial growth developed in their path except at the point of placement on the agar. The decontaminated nematodes and the fungus then were transferred to clarified agar plates. After 3-4 months of incubation, the developing nema colony again was transferred to fresh agar plates for increase. Bacteria were not observed in any of these subcultures.

In order to inoculate fungus and tissue cultures with masses of uncontaminated nemas, blocks of agar, containing nemas feeding at the margins of fungus colonies, were transferred to sterile potato-dextrose agar plates. After such a transfer, the nemas moved away from the agar blocks into the surrounding sterile medium and could be transferred singly or in groups, with very little chance of contamination. By this procedure, cultures of 115 species of fungi; *in vitro* potato, carrot, clover, and tobacco callus tissue; and tomato and clover roots were inoculated with uncontaminated nematodes. After a suitable incubation period, index numbers were assigned to indicate the relative amount of reproduction in each culture.

Material for histological studies was taken from infected tubers obtained directly from the field and from storage. Segments of diseased tuber tissues of the Katahdin variety, displaying various stages of infection, were killed and fixed in boiling 10% formalin, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Serial sections 20μ thick were cut with a rotary microtome, mounted on glass slides, and stained with Conant's quadruple stain (11).

Inoculations were carried out in the greenhouse on several possible hosts. The plants were grown in compost or Plainfield sandy loam soil which had been sterilized by autoclaving at 15-lb pressure for 1-3 hours. Air temperatures were maintained at 21-23°C. Inoculations were made 2-4 weeks after emergence of the plants. Nemas used for inoculum were removed from fungus or callus tissue cultures by the Baermann funnel method. Portions containing the desired number of nemas in water suspension were added to the top 2 in. of soil where roots or rhizomes were usually abundant. Water was added daily to prevent drying. Spore suspensions of fungi were prepared and introduced into the soil in the same manner. In cases where plants were inoculated with nemas or fungi or both, the nemas had been reared on callus tissues to ensure pure inoculum. Disease readings were made 3-4 months after inoculation. Disease index numbers were assigned to infected potatoes using a modification of the method designed by Walker, *et al.* (16) for potato scab. For other plants, readings were based on presence or absence of feeding and reproduction of the nema. Nemas were reisolated from infected plants for morphological comparison with nemas from the parent colonies and from naturally infected tubers.

Results.—Sixteen pure colonies of *D. destructor* were obtained from 190 gravid females picked from infected potato tissues and placed individually on cultures of fungi and callus tissues. Comparisons were made between 10 mature females from each of these colonies and known specimens of the nematode from potato. No differences were found in length a, b, and c proportions of de Mann (14); incisures of the lateral field; position of the deirid; oesophageal overlap; and shape of the tail terminus. Pathogenicity of 11 of these isolates also was compared on duplicate plants of the Chippewa variety inoculated at the rate of 5,000 nemas/plant. Two plants were inoculated with nemas removed directly from naturally infected tubers for comparison; 2 additional plants were used as noninoculated controls. Disease readings were taken after 4 months as previously described. Infected tubers exhibiting typical symptoms were recovered from all inoculated plants. Nemas recovered from these tubers showed no distinguishable differences between the 11 isolates, their parent colonies, and nemas from naturally infected tubers.

Attempts also were made to start colonies of *D. destructor* from 121 single-egg isolations. After 2-3 weeks of incubation, all nemas observed were adult or pre-adult females. Even after 3-4 months, however, there was no evidence of reproduction.

Behavior on fungi.—The ability of *D. destructor* to feed and reproduce on 115 species of fungi, isolated

mostly from hardwood soils of Northern Wisconsin and from decaying potato tubers, was studied. Uncontaminated nematodes from 1 of the pure colonies were introduced to duplicate cultures of each fungus at a rate of 50 nemas/culture. After 9 weeks of incubation at room temperature, index numbers were assigned as follows: 0—no apparent reproduction, 1—slight reproduction (100-1,000 nemas/culture), 2—moderate reproduction (1,000-10,000 nemas/culture), 3—good reproduction (more than 10,000 nemas/culture). Of 115 species of fungi inoculated, 64 were found satisfactory for development and reproduction of the nematode. These represented 40 genera, 8 orders, and all classes of the higher fungi.

Feeding and reproduction of *D. destructor* on duplicate cultures of 115 species of fungi was as follows:

Fungi	Index of Nematode Reproduction on Culture A	B
Phycomycetes		
Mucorales		
Absidia butleri Lendner	1	1
A. glauca Hagem	0	0
A. spinosa Lendner	0	0
Rhizopus nigricans Ehrenberd	0	0
Cunninghamella elegans Lendner	0	0
Haplosporangium sp.	0	0
Mortierella sp.	0	0
Mortierella sp.	1	0
Mucor hiemalis Wehmer	0	0
Zygorrhynchus vuilleminii Namyslowski	2	2
Peronosporales		
Phytophthora infestans (Mont.) de Bary	0	0
Pythium graminicola Subraminiam	0	0
P. irregulare Buismar	0	0
P. arrhenemanes Drechsl.	0	0
P. rostratum Butler	0	0
P. splendens Braun	0	0
P. pariocandrum Drechsl.	0	0
P. ultimum Trow	0	0
Saprolegniales		
Aphanomyces euteiches Drechsl.	0	0
Ascomycetes		
Plectascales		
Aspergillus flavipes Bainier & Sartory	1	1
A. fumigatus Fresenius	1	1
A. niger van Tieghem	0	0
A. terreus Thom	1	0
Penicillium funiculosum Thom	1	0
P. implicatum Biourge	2	2
P. janthinellum Biourge	0	0
P. javanicum Szilvinyi	3	3
P. liliacinum Thom	3	3
P. megalosporum Berk. & Br.	3	3
P. nigricans Bainier	3	3
P. purpurogenum Stoll	3	3
P. raistrickii G. Smith	1	1
P. restrictum Gilman & Abbott	3	3
P. roseo-purpureum Dierckx	3	3
P. simplicissimum (Andemans) Thom	3	3
P. stoloniferum Thom	3	3
P. thomii Maire	2	2
P. variable Sopp	0	0
Eurotiales		
Emericellopsis sp.	1	0
Thielavia basicola Zopf.	0	0
Hypocriales		
Gibberella zeae (Schw.) Petch	2	2
Sphaeriales		
Chaetomium indicum Corda	3	3
C. subterraneum Swift & Povah	3	3
Sporormia leporina Niessl	3	3
Basidiomycetes		
Agaricales		
Agaricus campestris Fr.	3	3
Armillaria mellea (Fr.) Quel	3	3
Deuteromycetes		
Moniliales		
Acrostalagmus sp.	1	1
Acrostalagmus sp.	2	2
Alternaria solani (Ell. & Mart.) L. R. Jones & Grout	2	2
Alternaria tenuis Nees	2	2
Alternaria tenuissma (Fr.) Wiltshire	2	2
Botrytis sp.	1	1
Botrytis sp.	0	0
Cephalosporium sp.	3	3
Cephalosporium sp.	3	3
Cephalosporium sp.	2	2
Cephalosporium sp.	2	2
Cephalosporium sp.	2	2
Cephalosporium sp.	0	0
Cephalosporium sp.	2	2
Cephalosporium sp.	2	2
Cephalosporium sp.	1	0
Cylindrocarpon sp.	3	3
Species of Dematiaceae	3	3
Species of Dematiaceae	2	2
Species of Dematiaceae	3	3
Fusarium oxysporum Schlechtendahl	1	1

	Index of Nematode Reproduction on Culture	
	A	B
F. oxysporum f. *pisi* race 1 (Linford) Snyder & Hansen	0	0
F. oxysporum f. *pisi* race 2 (Linford) Snyder & Hansen	0	0
F. oxysporum f. *lycopersici* (Sacc.) Snyder & Hansen	2	2
F. oxysporum f. *niveum* (E. F. Sm.) Snyder & Hansen	2	2
F. roseum (LK.) Snyder & Hansen	3	3
F. solani (Mart.) Sacc.	1	1
F. solani var. *pisi* (F. R. Jones) Snyder & Hansen	2	2
Fusarium sp.	1	1
Fusarium sp.	3	3
Heterosporium terrestre Atkinson	3	3
Hormodendrum sp.	2	2
Mesobotrys sp.	2	2
Myrothiciella sp.	3	3
Myrothecium gramineum Lib.	2	2
Myrothecium striatisporum Preston	1	1
Myrothecium striatisporum Preston (small strain)	1	1
Myrothecium verrucaria (Alb. & Schw.) Ditmar	3	3
Papularia sp.	1	1
Papularia sp.	2	2
Scopulariopsis sp.	2	2
Spicaria elegans Corda	3	3
Spicaria sp.	3	3
Sporotrichum sp.	3	3
Stachybotrys sp.	0	0
Stachybotrys sp.	1	0
Species of Stilbaceae	3	3
Thielaviopsis sp.	2	2
Tilaclidium sp.	2	2
Torula sp.	1	1
Oidiodendron nigrum Robak	3	3
Trichoderma viride Pers. ex. Fr.	3	3
Verticillium albo-atrum Reinke & Berth. (from tomato)	1	1
Verticillium albo-atrum Reinke & Berth. (from potato)	3	3
Vigaria sp.	2	2
Volutella roseola Cooke	0	0
Sphaeropsidales		
Diplodia zeae (Schw.) Lev.	2	2
Coniothyrium sp.	2	2
Coniothyrium sp.	3	3
Phoma conidiogena Schnegg	3	3
Phoma lingam (Fr.) Desm.	2	2
Phoma sp.	1	1
Pyrenochaeta sp.	1	0
Mycelia Sterilia		
Papulospora sp.	3	3
Rhizoctonia solani Kühn	0	0
Species of Mycelia Sterilia	3	3
Species of Mycelia Sterilia	1	1
Species of Mycelia Sterilia	3	3
Species of Mycelia Sterilia	3	3

Reduction in growth, due to nema feeding, was observed in many of the fungus isolates (Fig. 2). Curds, consisting of a mass of nemas, were formed later in the dishes after the fungus colony had ceased to develop and the culture had begun to dry.

Behavior on callus tissue.—Undifferentiated callus, growing *in vitro*, also was a satisfactory medium for rearing this nematode. Potato, carrot, clover, and tobacco callus tissues were grown on modified White's medium in 6-oz medicine bottles. The tissues were allowed to develop for 10-14 days at room temperature; 50 uncontaminated nematodes from 1 of the pure colonies were introduced into each culture. At least 10 cultures each of the above listed tissues were inoculated; 2 each were controls. The nematode reproduced in large numbers, up to 200,000/culture, on the callus tissues after 4 months of incubation at room temperature. Growth of the tissues was greatly reduced by nematode feeding (Fig. 2). The nematode failed to multiply on excised clover and tomato roots cultured in the same manner.

Sebago potato plants growing in 8-in. pots were inoculated with 0, 100, 200, 400, 800, 1,600, 5,000, and 10,000 nemas from a pure colony. Three pots were used for each treatment. After about 4 months, practically all tubers were infected in those pots receiving 5,000 or more nemas. Only 1 infected tuber was found in all of the treatments receiving less than 5,000 nemas.

The pathogenicity of 11 isolates, originating from single females and reared on *Oidiodendron nigrum* Robak, was tested on Chippewa potatoes growing in 8-in. pots. Sterilized soil was used and 5,000 nemas of each isolate were added to duplicate plants. For comparison, a replicate treatment used nemas removed directly from naturally infected tubers obtained from the field. After 4 months, nemas were reisolated from infected tubers and compared. No distinguishable differences were observed in size or morphology between any of the colonies, or their parent cultures maintained during the same period on the fungus culture, or the nemas directly from field-infected tubers. All isolates were pathogenic.

To determine the extent to which secondary organisms, mainly fungi, are associated with the

intensity of tuber symptoms, 4 series of experiments were initiated. In the first 3 series, potato plants were grown in 8-in. pots containing sterilized compost soil; in the fourth series, galvanized iron cans measuring 12 x 12 x 10 in. were used. Two fungus isolates (*Oidiodendron nigrum* and *Fusarium* sp.) were introduced at a rate of 25-ml spore suspension/plant. Uncontaminated nematodes, which had been removed from clover and tobacco callus, were introduced at a rate of 5,000 nemas/plant. Both fungi and nemas were introduced separately and in combination. In series 1 and 2, inoculations were made 7 weeks after the potatoes were planted; in series 3 and 4, inoculations were made 4 weeks after planting. Disease readings were taken 3.5 months after inoculation. Index numbers, based on severity of symptom expres-

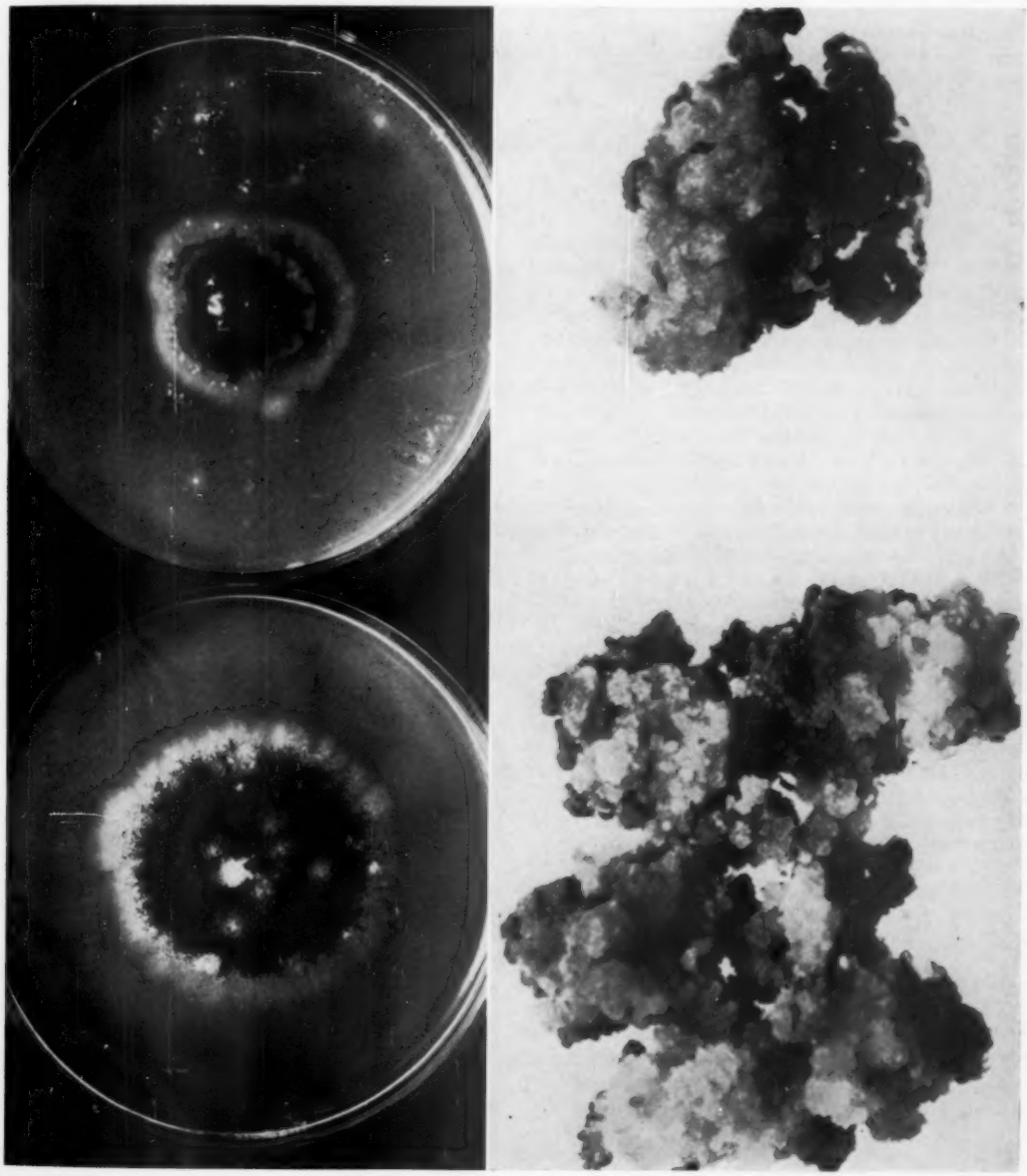

Fig. 2. Effect of *Ditylenchus destructor* on growth of *Oidiodendron nigrum* and carrot callus. Top: inoculated. Bottom: uninoculated.

TABLE 1. Effect of fungi on tuber rot incited by *Ditylenchus destructor*

Series	Treatment	Replications	Avg. no. tubers/plant	Avg. total wt. tubers/plant	Avg. of disease indices
1	Nema + *Fusarium* sp.	5	3.6	not taken	41
	Nema alone	5	3.4	not taken	20
	Fusarium sp. alone	5	3.2	not taken	0
	Uninoculated	5	3.6	not taken	0
2	Nema + *Oidiodendron nigrum*	5	3.6	not taken	42
	Nema alone	5	3.2	not taken	29
	O. nigrum alone	5	3.8	not taken	0
	Uninoculated	5	3.6	not taken	0
3	Nema + *O. nigrum*	4	2.75	43.3 g	21
	Nema alone	4	4.25	98.5 g	33
	O. nigrum alone	1	4.0	127.0 g	0
	Uninoculated	1	5.0	183.0 g	0
4	Nema + *O. nigrum*	4	4.25	47.5 g	16
	Nema alone	4	6.75	220.0 g	20
	O. nigrum alone	1	4.0	65.0 g	0
	Uninoculated	1	2.0	26.0 g	0

sion, were assigned as follows: 0—healthy, 1—light infection, 2—moderate infection, 3—severe infection. Total weights of tubers produced by plants in series 3 and 4 also were recorded.

Tuber symptoms were present when the inoculum consisted of nematodes alone, but were much more severe when the inoculum contained a fungus as well as the nematode (Table 1, series 1 and 2). The data from series 3 and 4 might indicate conflicting results had only disease indices been assigned. In these series disease indices were greatest when the plants received the nematodes alone, but yield was reduced when the inoculum contained a fungus as well as the nematode. These results may be explained by the fact that inoculum was added to plants in series 3 and 4 just 4 weeks after planting. The first tubers formed were completely destroyed on plants receiving a fungus as well as the nematode, as shown by the presence of empty potato shells.

Several plants other than potato were tested for susceptibility to the potato rot nematode. All nemas used in these tests were removed from carrot or tobacco callus cultures. The colony selected had undergone 3 successive single female isolations and was pathogenic to potato, as shown by inoculation and reisolation comparisons. Carrot, onion, garlic, clover, alfalfa, mint, parsnip, turnip, red beet, sugar beet, bulbous iris, narcissus, gladiolus, radish, and sweet potato were grown in 6-in. pots containing steam-sterilized soil. Four replications of each plant were used; 3 each were inoculated at the rate of 5,000 nemas/pot. Disease readings were taken after 4 months and nemas were recovered from infected plants for morphological comparisons. Carrot (*Daucus carota*), red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), parsnip (*Pastinaca sativa*), radish (*Raphanus sativus*), sweet potato (*Ipomea batatas*), and bulbous iris (*Iris* sp.) were susceptible to the potato rot nematode. Alfalfa, parsnip, and radish have not previously been reported as hosts. With the exception of alfalfa and clover, the symptoms produced on these hosts were similar to symptoms on potato. Large, open, necrotic lesions were present on the subterranean parts of infected plants and were restricted to the cortical tissues. Rotted crowns and dwarfing occurred in infected alfalfa and clover.

Susceptible host plants growing in nature influence the body proportions of *D. destructor* (9). So, nemas recovered from each of the above hosts were compared with nemas from the parent colony growing in culture and from naturally infected tubers. The a, b, and c proportions of de Mann (14) were selected as criteria and only mature gravid females were used in these comparisons. No significant differences were found between the body proportions of the nemas recovered from any of the hosts or from the parent colony maintained on cultures of fungi and callus tissues. The slight variations observed were within the measured limits found repeatedly in populations taken directly from naturally infected potato tubers and from numerous cultures of fungi and callus tissues.

Initial infections on young tubers were characterized by the presence of small chalky areas under the periderm of the tuber. Free-hand sections made from these tubers showed many collapsed cells in the chalky areas whereas other cells appeared undamaged. Cell-to-cell movement of nemas was observed in tuber tissue. A nematode completely enters a cell, then places its anterior end in a position vertical to the wall on 1 side of the cell and its posterior end flat against the wall on the opposite side. By this means, the nema gains enough leverage or traction to distend and quickly puncture the cell wall. By repeating this process, it moves rather easily from cell to cell. The puncturing of the cell wall apparently is a combination of mechanical force and action of the stylet. Although feeding was not observed in tuber tissue, the action of the stylet in penetrating the hyphal wall of fungi has been observed repeatedly.

Free-hand sections made of advanced stages of infection showed normal tissues near the margins of the invaded areas. Infected portions, however, were

difficult to observe under the microscope because of masses of moving nematodes, oligochaetes, and mites. Fungus hyphae and bacteria also were abundant in many of the advanced stages.

Histopathology.—Tuber tissues, representing various stages in disease development, were processed by the histological methods previously described. Examination of young infections indicated that nemas, after penetrating the periderm, traveled through the outer 4-5 cortical cells and started to feed. Many of the cells in these areas collapsed, whereas others remained intact, giving such areas a chalky appearance when observed with the unaided eye. The starch content of cells immediately adjacent to the feeding area was greatly reduced; there was a tendency for the nuclei and cytoplasm of these cells to become oriented on the side of the cell closest to the feeding area. These responses often extended several cells beyond the point of feeding.

As feeding continued and the lesion increased in size, a definite cavity was formed. Remnants of cell walls and a few starch grains were present in these cavities. Orientation of the nuclei and cytoplasm, as well as starch reduction, were more pronounced and extended farther into the surrounding tissue.

As nema populations increased, a series of cavities was formed. The nemas apparently fed in 1 area, produced a cavity in the tuber tissues, then spread out into the adjacent tissues, producing additional hollows until a reticulate system of tunnels and cavities resulted (Fig. 1).

Invasion of these areas by secondary organisms, mostly fungi, mites, and bacteria, suggested a continuation of disease development. In lesions where fungi predominated as secondary invaders, great numbers of nematodes usually were found. When mites and bacteria also were abundant in these lesions, however, few nemas were present.

Final stages in disease development were characterized by rifts in the periderm and large, open, necrotic lesions. The formation of wound tissue around older lesions indicated that disease progression had stopped.

Discussion.—The potato rot nematode was successfully reared in the laboratory under aseptic conditions on a great number of fungi. The techniques used provided a means of direct microscopic observation of the actual feeding habits and of all developmental stages in the life cycle of the nematode. Most of the fungus isolates from soil and decaying tubers were suitable hosts. Feeding and reproduction did not occur on *Penicillium janthinellum*, for example, but developed in great numbers on *P. javanicum* and 8 other species belonging to this genus. This phenomenon was not uncommon among the fungi tested, since preference was observed within and between several different genera. This evidence also suggests that a wide variety of fungi may play an important role in the survival of this nematode in nature.

Aseptic callus tissue cultures also afforded a technique by which large populations of *D. destructor* were developed from single gravid females under a rigidly controlled environment. The results suggest that other plant parasitic nematodes might be reared on this medium. Root cultures grown in the same medium were unsatisfactory since the nematode was unable to penetrate the growing roots. Nemas were attracted to the roots in large numbers and were observed attempting to penetrate root cells, as evidenced by rapid stylet action. Either the cell walls were too thick or the nemas did not have sufficient leverage or traction in the type of medium used. Damage to potato roots was not observed in nature or in the inoculated plants in the greenhouse.

Pathogenicity of the nematode was maintained for 4 years of continuous laboratory culture on fungi and callus tissues. During this period, there was no evidence of variation in structure or morphology of any of the collections, indicating considerable stability of this species. Specific host-plant preference also was maintained during this time.

Fungi played an important role in tuber breakdown. Addition of a fungus to the nematode inoculum increased the intensity of tuber damage under greenhouse conditions. Large numbers of nematodes were invariably present in those lesions in which concentration of fungus hyphae was greatest. On the other hand, usually not more than 5-10 nemas were present in small chalky lesions in the absence of a fungus. Lesions in which both nemas and fungi were present continued to develop in tubers stored as long as 4 months at 5°C. Symptoms were present, similar to those of several common types of dry-rot breakdown in stored tubers usually associated with mechanical damage. Bacterial wet rot was sometimes associated with heavy nematode damage, but few nemas were present in such tubers. There was no evidence that nemas spread from infested to healthy tubers in storage.

The behavior of *D. destructor* in nature is erratic. In Wisconsin, the earliest infected tuber ever found was on August 7, approximately 70 days after planting. Frequently, by harvest in mid- or late-September, all of the tubers in a heavily infested area were unmarketable. Such areas may be as large as 6 acres and may persist for several years under either continuous potato culture or a 3-year rotation with oats and red clover. Small infested spots involving 1 to a few infected tubers usually disappeared after 1 season. New infestations of this type, however, sometimes appeared in other areas of the same field in subsequent years. Apparently, a very low population of this nema is sufficient to maintain an infestation from season to season. Critical examination of many soil samples taken in the spring and summer from well-established infestations repeatedly failed to reveal the presence of this species. Likewise, the nema was rarely found except superficially on weed hosts or other crops grown in a rotation. Since numerous soil fungi are hosts, it is possible they serve as primary hosts and that the

nemas move from these hosts to young tubers where the population builds up rapidly during the period of tuber development.

The host range of *D. destructor* is obviously incomplete. Although 3 previously unreported higher plant hosts were susceptible, it is felt that the number will increase. Care must be exercised, however, in determining the susceptibility of a host. Direct damage caused by the nematode should be present and accompanied by host reaction and nematode reproduction. Certain inoculated plants tested in the greenhouse had nematodes on them or superficially present around the crown. No evidence of feeding or good reproduction could be found and such plants were not considered susceptible. The presence of numerous nematodes in or around the crown of certain plants suggests they may have been feeding on hyphae of 1 or more of the fungi present.

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PATHOGEN VARIABILITY AND BREEDING VERTICILLIUM WILT RESISTANT STRAWBERRIES

R. S. Bringhurst, Stephen Wilhelm, and Victor Voth

Department of Pomology, University of California, Davis.

Respectively, Associate Professor of Pomology, University of California, Davis; Professor of Plant Pathology, University of California, Berkeley; and Associate Specialist, Pomology, University of California, Davis.

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SUMMARY

The reactions of 18 strawberry clones differing in susceptibility to *Verticillium* wilt, including the Lassen, Shasta, and Solana varieties, were evaluated in individual glasshouse tests against 6 fungus isolates, including 3 pathogenic to tomatoes and 3 nonpathogenic to tomatoes. The reactions were related to appropriate field tests on soil fumigated with chloropicrin-methyl bromide and to previous field tests on unfumigated soil. Tests were made 1956-60. Composite inoculum was used in most field tests. Except for the highly susceptible Lassen, Shasta, and Solana varieties, the strawberry clones did not react uniformly to all of the fungus isolates. Fungus isolates differed highly significantly in average virulence. Even when highly resistant reactions were evident, the fungus invaded a high percentage of the individual test plants concerned, suggesting a lower level of susceptibility rather than resistance *per se*. Since individual tests of large numbers of fungus isolates are not practical, tests of mixtures of spores from a number of representative isolates are recommended for breeding and inheritance studies. With adequate inoculation procedures and scoring systems, field tests with small plots (about 10 plants) and few replicates (about 3) can measure differences in strawberry clone reactions accurately and reliably.

Verticillium wilt caused by *Verticillium albo-atrum* Reinke & Berth. has been an important disease of the strawberry (*Fragaria* sp.) in California for many years (6). More recently, it has been recognized as a problem in most of the important strawberry-growing areas of the world. Wilhelm (8) has discussed the pertinent literature.

Well-known resistant varieties, such as Sierra, Marshall, and Blakemore, are not immune. Varieties designated "resistant" have exhibited slight-moderate susceptibility in different tests (2, 3, 6, 7, 8). Much of the observed variability in resistance in single-host clones is likely due to fungus strain differences and seasonal environmental fluctuations. This was strongly indicated by the results of our work in 1956-59.

During 1960, experiments were conducted, in glasshouse and field, on the influence of fungus strain differences on the incidence and severity of wilt in commercial strawberry clones originating from the University of California breeding program and classified as resistant or susceptible. The 1960 experiments also were designed to determine whether resistance to a number of fungus strains can be ascertained by a composite test involving inoculum of mixtures of different fungus strains, or whether tests must be strain-by-strain. This paper reports the results of the 1960 experiments, with the experiments of 1956-59 summarized as a background.

MATERIALS AND METHODS.—Experiments in the field were conducted at the University of California Deciduous Fruit Field Station, San Jose, a site representative of the Santa Clara Valley strawberry production area. Tests prior to 1959 were made on unfumigated land that for at least 10 years had been planted to tomatoes or wilt-inoculated strawberries and was heavily infested with *V. albo-atrum*. The 1959-60 plantings were made on the same land after fumigation with 340 lb/acre of a 3:2 mixture of chloropicrin and methyl bromide. Immediately after fumigation, the soil was covered with polyethylene sheeting for 24 hours. The soil was fumigated to reduce soil-borne fungus populations pathogenic to strawberries, which had complicated plant reaction to *Verticillium* in previous years. Inclusion of uninoculated susceptible plants in the experiments established that the fumigation almost completely controlled the soil-borne pathogens. Field evaluations were supplemented by the glasshouse tests during 1960.

Plants and planting.—All strawberry plants were harvested from University nurseries in December of the year preceding the experiment, packed in thin polyethylene bags, and stored at -2°C until planting time. Field plantings were set in double rows on raised beds employing hill-system culture.

The field-plots contained 12 plants per entry per replicate except in 1956-57 (respectively, 50 and 25 plants) and in a small 1960 experiment (6-plant plots). There were 3 replicates for each entry in 1959-60. Only the control entries (Shasta, Lassen, and certain resistant clones) were replicated in 1956-58.

The 1956, 1957, 1959, and 1960 field plantings were established between March 21 and April 1, and the 1958 planting on April 23.

The 1960 glasshouse experiment was started in mid-February. Nine plants/entry were used with 3 assigned to each of the 3 replicates. Standard 4-in. plastic pots were used for 2 replicates, and similar clay pots for the third. The soil was U.C. Mix C (1), with hoof and horn grist as the basic fertilizer.

Fungus isolates.—Six different isolates of *Verticillium* from Wilhelm's laboratory were used individually in the 1960 glasshouse experiments. Three isolates (S2, V9, S41), pathogenic to both strawberries and tomatoes, were designated as tomato-positive strains. Three isolates (S10, S36, S112), pathogenic to strawberries but nonpathogenic to tomatoes, were designated as tomato-negative strains. In cultural characteristics, these strains are indistinguishable. All *Verticillium* isolates used, except V9, originated from strawberries. A common source of tomato-negative strains in nature is the nightshade weed (*Solanum sarachoides* Sendt) (10).

Two of the strains, 1 from each pathogenicity group, originated from strawberry-growing areas in San Jose (S2, S112), 2 from Watsonville (V9, S10), and 2 from Los Angeles (S41, S36). All isolates had been in active laboratory culture or in a dormant state of dried growth on tomato stem pieces for up to 5 years. For the preparation of inoculum, each culture originated from a single conidium, and only colonies of the conidial (C) type (4) were retained.

For the 1960 field experiments, 2 composite types of inoculum were used; 1 combined the 3 tomato-positive isolates (S2, V9, S41); the other combined the 3 tomato-negative isolates (S10, S36, S112). In 1959, only isolates V9 and S112 were used (individually). In 1958, 2 composite types of inoculum were used; 1 included V9 and a second tomato-positive isolate; the other included S112 and a second tomato-negative isolate. In 1956-57, a single composite inoculum was used including V9 and S112 plus 1 tomato-positive and 1 tomato-negative isolate.

Inoculation.—Plants were washed. Then, each group of 10 or 12 was immersed in a spore suspension consisting of the spore yield from a 2-week-old PDA slant culture in a 20 x 200-mm tube. Aseptic precautions were used during the inoculation and planting operations. Plants were inoculated within 2 days of planting; except in 1959, when inoculated plants were stored 10 days at 0°C immediately prior to planting.

Scoring system.—Beginning about 50 days after planting, each plant was scored on the basis of symptoms. Scorings were made at 2-4-week intervals throughout the growing season, as follows: 0, *no evident symptoms;* 1, *slight starting symptoms,* in which outermost leaves became flaccid and showed marginal and interveinal chlorosis; 2, *general symptoms with prospect of surviving* in which extensive collapse and drying of outer leaves occurred, but strong crown growth continued; 3, *general symptoms with prospect*

Fig. 1. 1956 San Jose wilt nursery in November. Contrast the highly resistant reaction of clone 53.1-21, right foreground, with the highly susceptible reaction of 53.9-1, left foreground, in which all plants are dead.

of dying, same as number 2 except that new crown growth ceased and before death the plant assumed a distinctly stunted flattened appearance; and 4, *dead plant*. The cumulative scores were summed for each plot, and the total divided by the number of plants in the plot. The value obtained, representing the average cumulative score/plant, was the original datum used for comparing selections, fungus strains, and the interactions in the analysis of variance and Duncan range tests. Thus, a plot scored 6 times in which the plants were all dead at the first scoring would have a cumulative average score of $4 \times 6 = 24$; whereas a plot scored 6 times in which none of the plants manifested symptoms would have a cumulative average score of $0 \times 6 = 0$. The scores reflected the relative rapidity with which plants succumbed, as well as the final observed condition of the planting at the end of the season. Fig. 1 is a photograph of the 1956 plots at the end of the season.

In presenting the data (Fig. 2-6), the numerical scores are accurately represented by the horizontal dimensions of the scalariform diagrams. Statistical significance is shown by alphabetical letters; differences are significant (1% level) only if the represented means have no lower case letters in common.

RESULTS.—*Glasshouse tests with individual fungus strains.*—Fig. 2 presents results of the 1960 glasshouse experiment, in which 18 strawberry clones were tested individually with 6 fungus isolates. The first column represents the average reaction of the selections in ascending order of susceptibility over all fungus strains. Each of the other 6 columns represents the individual reaction of each of 18 selections to 1 of the 6 fungus strains.

According to the analysis of variance, differences in the disease ratings were highly significant among the strawberry clones over all fungus strains (average reaction, Fig. 2) and among the strawberry clones within each fungus strain. The letters designate significant differences for each column.

The average reaction of all strawberry clones to each fungus isolate is represented in the bottom row, with increasing order of virulence from left to right. According to the analysis of variance, differences in virulence among the fungus strains were highly significant. The highly significant differences were due partly to the low virulence of strain V9, to which only 3 clones were highly susceptible (Shasta, Lassen, and Solana), and partly to the high virulence of S36, to which 10 of the 18 clones were highly susceptible.

Strawberry clone 52s11-2 appeared virtually immune to all of the fungus strains except S36, to which it was definitely susceptible. In all previous tests, 52s11-2 was in 1 of the highest resistance classes. In the 1956

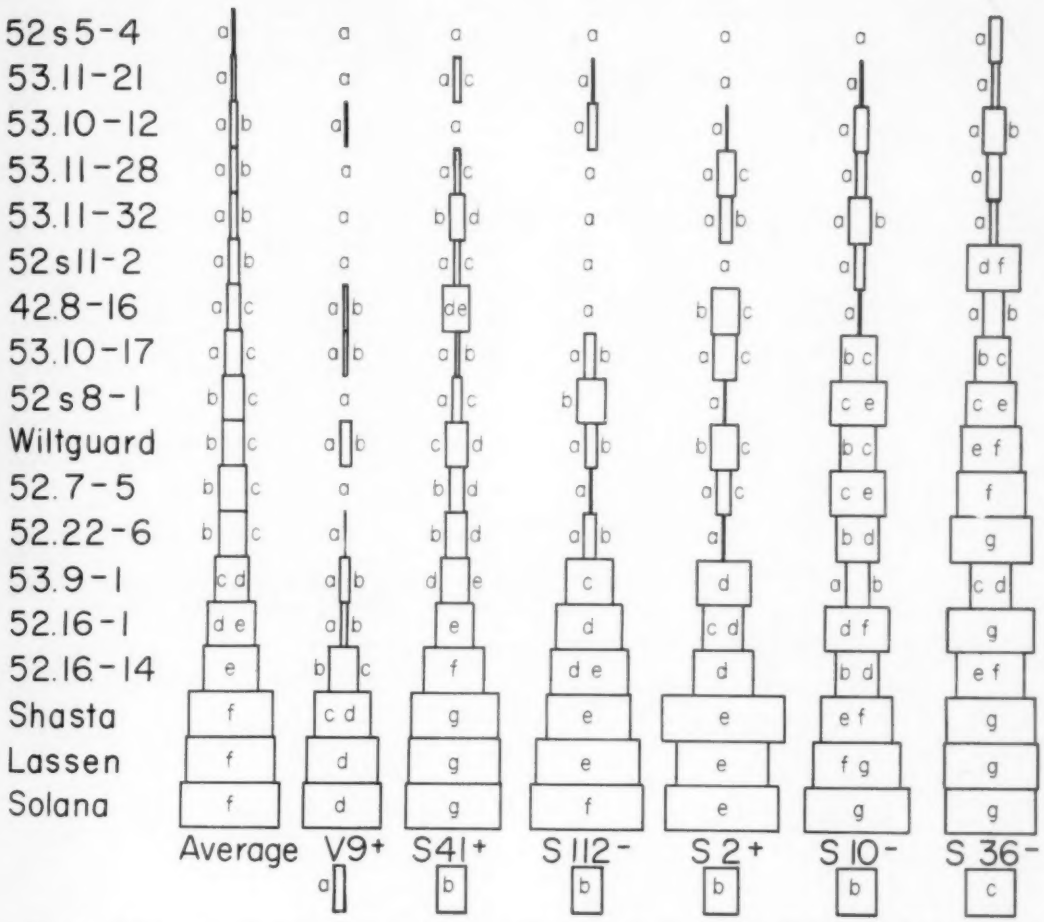

Fig. 2. Rating of 18 strawberry clones, ranked according to average reaction in 1960 individual glasshouse tests against 3 tomato-positive (+) and 3 tomato-negative (−) fungus isolates. Breadth of individual blocks represents cumulative symptom scores. Clonal reactions for each column differ significantly (1% level), if they have no lower case letters in common; only the first and last applicable letters are given. Average fungus isolate virulence and significance represented bottom, left to right. Greatest represented score (Shasta against S2+) 27.7; smallest, 0; maximum possible score, 48.

and 1958 tests (Fig. 3), it fell into classes 1 and 2, respectively. In the 1959 tests (Fig. 4), it ranked highest in resistance.

Glasshouse tests vs. field tests.—Field reactions to inoculation are probably similar to glasshouse reactions. In 1959, 12 of the selections used in the 1960 tests were included in individual field tests against fungus isolates V9 and S112 on soil fumigated with chloropicrin-methyl bromide. The results of the V9 field test are omitted, because there was no significant difference between the uninoculated checks and the plants inoculated with V9. This, the only inoculation failure in 5 years, may have been due to storage of the plants after they were inoculated. Fig. 4 compares the field (1959) and glasshouse (1960) reactions of 12 strawberry clones against *Verticillium* isolate S112. In general, the results indicate a close correlation between field and glasshouse tests.

Combined inoculum vs. individual tests.—The question of the type of evaluation required to provide a reliable basis for predicting the reaction of a given selection to a wide range of inoculum can be partially answered by comparing 1960 glasshouse results with 1960 field results. Appropriate groupings of the 1960 glasshouse data are compared with 1960 field data for the same 18 strawberry clones in Fig. 5. The first pair of columns compares the combined data of the glasshouse experiment with those of the field. The second pair of columns compares the combined glasshouse data for the tomato-positive strains used individually with the field data for the same clones inoculated with combined tomato-positive isolates. The third pair of

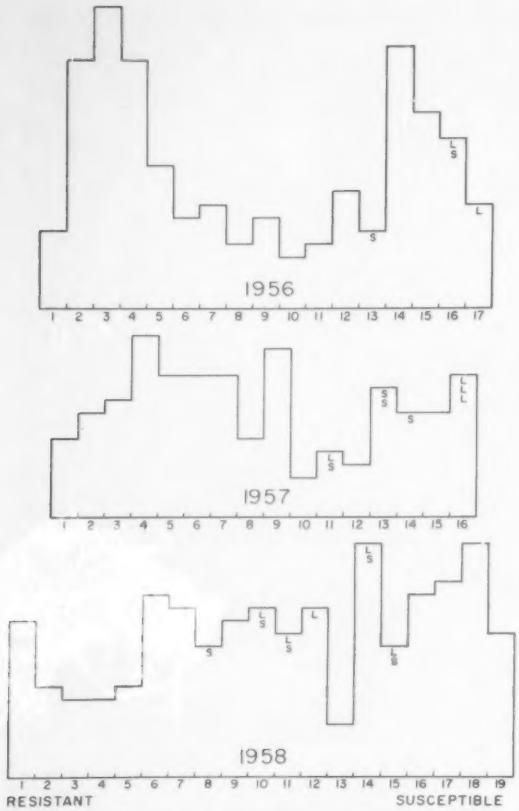

Fig. 3. Frequency distributions of strawberry clones in arbitrary reaction classes (1, 2, 3, *etc.*): 158 tested in 1956, 273 in 1957, and 214 in 1958. L and S respectively designate classes where Lassen and Shasta controls fell (4 each in 1956 and 1957, 2 each in 1958), approximating the range of high susceptibility for that year.

columns is the same comparison for the tomato-negative strains. In general, the field results were similar to those obtained in the glasshouse, as illustrated by the similarity in the truncate columns, although in almost every instance the symptoms were more severe in the field than in the glasshouse.

Discrepant reactions.—Clones such as 52s8-1 were markedly resistant to the tomato-positive isolates as a group, but exhibited significant susceptibility to the tomato-negative isolates. This may account for the inconsistent record of 52s8-1 in earlier tests. In 1956, it was judged resistant since it fell into class 1, whereas in 1958 it was judged susceptible since it fell into class 9 (Fig. 3). In the glasshouse test, clone 52s8-1 was definitely susceptible to fungus strains S10 and S36 (Fig. 2) and also was affected by S112. Thus, the cumulative score became great compared with those for 52s11-2 and Wiltguard, which in the individual tests were highly susceptible only to S36.

The Sierra variety was tested in an adjacent but separate field experiment in 1960 (Fig. 6) where it exhibited a reaction similar to that of clone 52s8-1 (Fig. 5). Like clone 52s8-1, Sierra was judged susceptible in the 1958 test since it fell into class 10 (Fig. 3).

Relative susceptibility of commercial clones.—The Lassen and Solana varieties are probably as highly susceptible as any strawberry clone in the University breeding program. Shasta may be a little less susceptible to certain fungus isolates (Fig. 2). In addition to the 15 clones compared with Lassen, Shasta, and Solana in the 1960 field tests (Fig. 5), 24 other clones of varying susceptibility were included. These 24 are compared with Lassen, Shasta, and Solana in Fig. 7. None was significantly more susceptible than the 3 highly susceptible varieties; however, clone 41.61-1 appeared significantly less susceptible to the tomato-negative fungus group than almost any other. Clone 43.1-2 manifested the most discrepant reaction to the 2 fungus groups. It was as susceptible as Lassen, Shasta, or Solana to the tomato-negative group, but as resistant as clone 41.61-1 to the tomato-positive group.

Resistant and intermediate reactors.—Over 700 different strawberry clones have been evaluated in these 5 years of experiments. Every clone in every field test manifested some symptoms, and none was

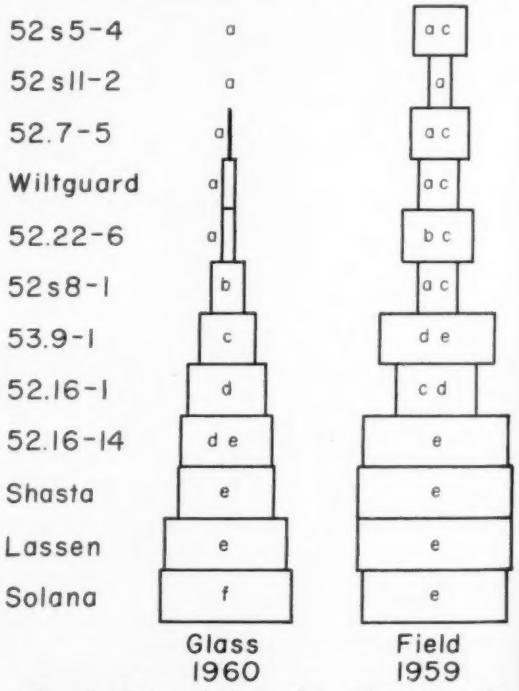

Fig. 4. Rating of 12 strawberry clones, comparing 1960 glasshouse test with 1959 field test against fungus isolate S112. Breadth of individual blocks represents cumulative symptom scores. Clonal reactions for column differ significantly (1% level) if they have no lower case letters in common; only the first and last applicable letters are given.

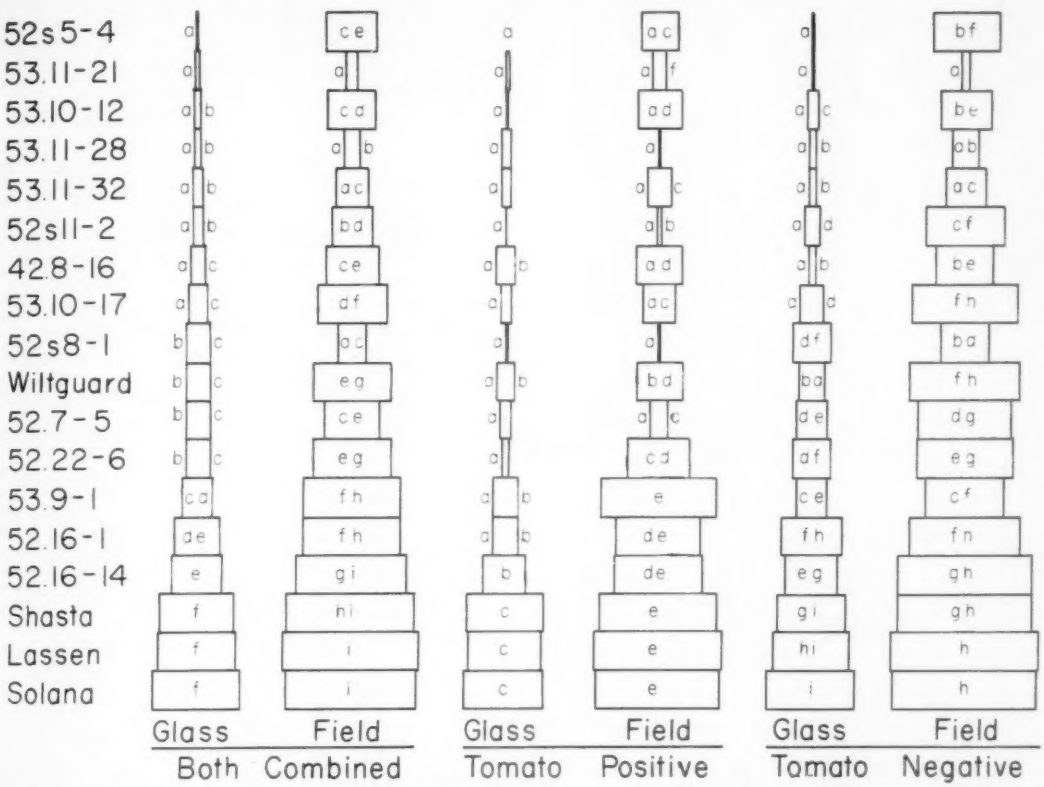

Fig. 5. Rating of 18 strawberry clones, ranked according to average reaction in 1960 glasshouse and field tests, comparing appropriately pooled glasshouse reactions with field reactions against indicated *Verticillium* inoculum groups (12-plant plots, 3 replicates). Breadth of individual blocks represents cumulative symptom scores. Clonal reactions for each column differ significantly (1% level) if they have no lower case letters in common; only the first and last applicable letters are given.

judged immune. When attempted, isolation of the pathogen was successful from petioles of plants of all resistant clones. In the 1958 group (Fig. 3), fungus isolations were made from the 21 most resistant strawberry clones. In all, isolations were attempted from petioles of 330 plants, 8-24 from each selection, and were 64% positive. Recoveries from the 21 most resistant clones ranged 25-100% of the individual plants of each clone. For example, the fungus was recovered from 17 of 22 plants of clone 53.11-21, and from 16 of 22 of 52s11-2. In November, when the 1958 experiment was terminated, these plants, for the most part infected by *Verticillium*, appeared symptomless and capable of performing satisfactorily for another season.

Most of the symptoms in inoculated resistant plants appeared early in the season. These intermediate reactors recovered, whereas susceptible plants usually died. This is illustrated in Fig. 8, in which the seasonal symptom scores for the highly susceptible varieties, Lassen, Shasta, and Solana, are compared with scores for the moderately resistant Wiltguard variety and the highly resistant clone 52s11-2. This test was conducted in the field in 1959 with fungus isolate S112. All plants of Lassen and all but 1 each of Shasta and Solana died before October 16 in that test. In contrast, though almost every plant of Wiltguard and 52s11-2 showed slight to severe symptoms during the season, none died. On the first 2 scoring dates, occasional plants of Wiltguard showed relatively severe symptoms, but by October 16 nearly all were essentially symptomless.

Performance of inoculated strawberry clones.—Plants from the 1956 and 1959 experiments were carried into the second year. Inoculated plants that appeared healthy at the end of the first year performed reasonably well the next year. In 1959 experiments, however, which were conducted on soil fumigated with chloropicrin-methyl bromide, yields of these resistant but infected plants were significantly less than for the uninoculated controls. The average reduction in yield for inoculated plants of each of 3 intermediate reactors in the 1959 tests was: 39.117-4, 40%; Wiltguard, 29%; and 52.22-6, 30%. Nevertheless, these yields would still have been commercially profitable. Performance of these clones contrasts

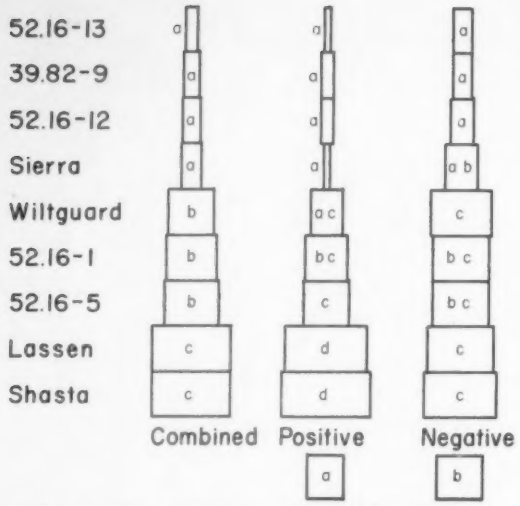

Fig. 6. Rating of 9 strawberry clones (comparing resistant Sierra), ranked according to average reaction in 1960 field test against tomato-positive and tomato-negative composite inoculum (6-plant plots, 2 replicates). Breadth of individual blocks represents cumulative symptom scores. Clonal reactions for each column differ significantly (1% level) if they have no lower case letters in common; only the first and last applicable letters are given.

sharply with that of the inoculated Lassen, Shasta, and Solana plants which in the above tests were dead and yielded nothing.

Discussion.—The fundamental objectives of these experiments were to determine first, the type of test that would permit a reliable estimate of the generalized reaction of a given strawberry clone to *V. albo-atrum*; and second, how differences in fungus isolates may influence ratings, and how testing can be standardized to obtain uniform results from year to year. Obviously, the questions are closely related.

Test plot size.—From the data presented we concluded that, for a given field test, resistant, intermediate, and susceptible clones can be separated with reasonable certainty with as few as 6 plants per entry per replicate and as few as 2 replicates, although at least 3 replicates and a minimum of 10 plants/plot probably should be used, depending on the number of entries. We assume inoculation procedures similar to ours. This conclusion is at variance with that of Baker and Voth (3) and Baker and Baker (2), obtained in tests at the same site, that 50-plant plots with 3-4 replicates were only of "fairly adequate" size to assess resistance of strawberry clones. They stated, however, that it would be helpful "if improved methods of inoculating the plants or preparing the soil" were employed. Both these conditions were met in our experiments, and a third important improvement also was included—the scoring system. Baker, *et al.* (2, 3) scored plants at the end of the season only, neglecting the intermediate reactors that manifested severe symptoms early but recovered completely as the season progressed. Repeated scorings are important because, under conditions of extreme moisture stress, plants will die that otherwise might perform satisfactorily. In our experiments, all symptoms were scored and cumulative scores were used for all comparisons.

Artificial inoculation.—Naturally infested soil will probably not give sufficiently uniform infection without an efficient system of artificial inoculation. Further, if investigations are conducted in the field, the soil should be fumigated with chloropicrin or chloropicrin-methyl bromide to ensure relatively uniform soil environment. Baker and Baker (2), using a relatively inefficient inoculation procedure on unfumigated soil in tests at the same San Jose site, rated the Shasta variety as having slight-medium infection, and Lassen as having medium-heavy infection, on the average. Further, when the original data of Baker and Baker (2) on Sierra, Shasta, and Lassen were compared by

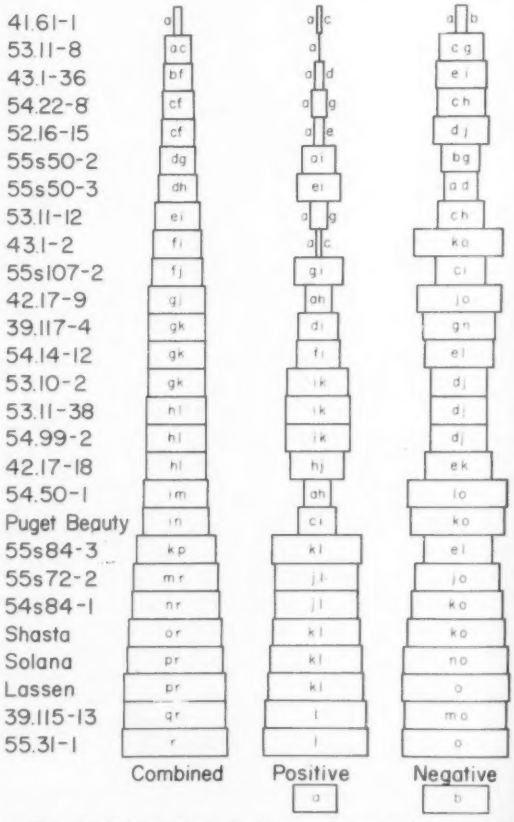

Fig. 7. Rating of 27 strawberry clones, ranked according to average reaction in 1960 field test against tomato-positive and tomato-negative composite inoculum (12-plant plots, 3 replicates). Breadth of individual blocks represents cumulative symptom scores. Clonal reactions for each column differ significantly (1% level) if they have no lower case letters in common; only the first and last applicable letters are given.

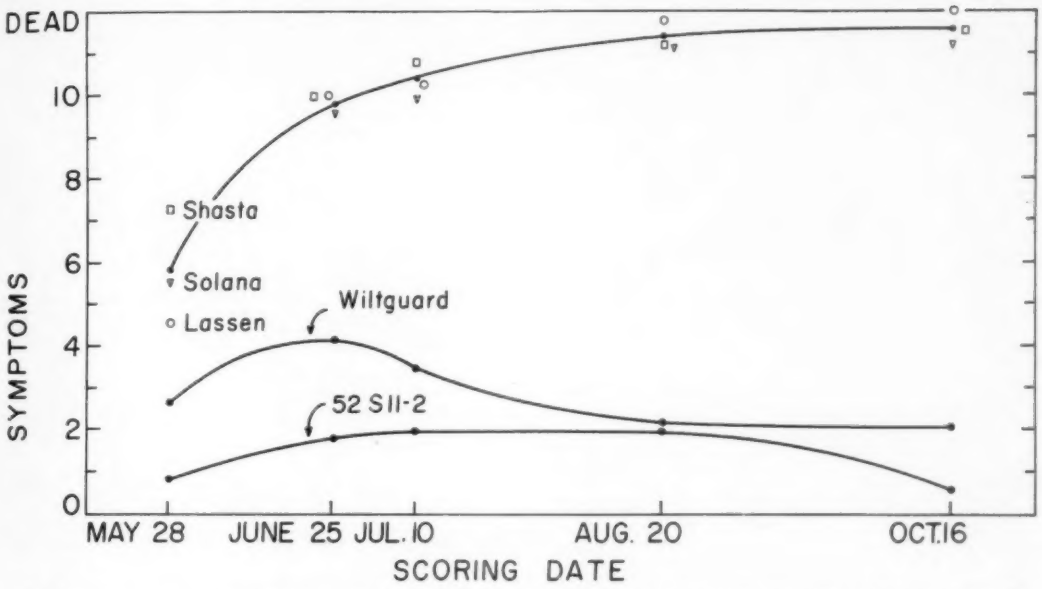

Fig. 8. Seasonal manifestation of symptoms (caused by *Verticillium* isolate S112), comparing the average reaction of 3 highly susceptible varieties (Shasta, Lassen, and Solana) with a moderately resistant variety (Wiltguard) and a highly resistant clone (52s11-2) in the 1959 field test on fumigated soil.

the Duncan range test, we found that, though Sierra was significantly different from Lassen at the 5% level, Sierra was not different from Shasta because Shasta was only slightly infected. In all of our experiments throughout 5 years, except for the single test failure in 1959, over 96% of the Shasta and Lassen plants were dead by the end of the first growing season. In our 1960 field tests, highly significant differences between Sierra and either Shasta or Lassen were measured in the field with only 2 replicates and (in that particular experiment) only 6 plants/plot (Fig. 6). This is consistent with the commercial behavior of Sierra plants, compared with that of Shasta or Lassen plants, exposed to natural infection in California. If natural infection is relied on for resistance tests, the size of the basic plots must be substantially increased. This adds greatly to the cost of testing the large groups of selections necessary in a breeding program.

Fungus strains.—Fungus strain differences must be considered in breeding *Verticillium*-resistant strawberries. The 6 isolates used were originally selected because of their virulence against the Shasta, Lassen, or similarly susceptible varieties. In these tests, Shasta, Lassen, and Solana have shown highly susceptible reactions similar to the original field reaction. Fungus isolate V9, included in all of the experiments reported here, was the standard fungus in resistance studies reported by Wilhelm (8, 9). In the 1960 glasshouse tests, the 3 highly susceptible varieties were highly susceptible in tests against isolate V9. In contrast to this, 8 of the 14 clones were resistant to V9, and highly susceptible to isolate S36 along with the 3 varieties.

Combined inoculum vs. individual tests.—Individual tests against large numbers of fungus isolates are not practical in a breeding program. The problem of obtaining a reliable generalized appraisal of the reaction of individual strawberry clones to a wide variety of fungus strains might be approached in either of 2 ways.

For the first approach, the most virulent strain available, such as S36, might be used alone as the screening fungus. In the 1960 tests, the greenhouse reaction of a given strawberry clone tested against S36 alone correlated quite closely with the field reaction of the same strawberry clone tested against the combined inoculum containing S36. Further testing is necessary before the single-fungus method can be recommended.

The second approach is to use composite inoculum composed of spores from a number of representative fungus strains such as the 6 used here. This approach offers the best possibilities now, although more testing is needed. If screened in this way, all of the strawberry clones would probably react to some degree, but by a cumulative system of scoring, the clones could be clearly classified on a statistical basis. Useful resistance could thereby be recognized easily.

Resistant clones often infected.—Invasion of resistant plants may occur; but, as Newton and Van Adrichem (5) pointed out, this is not necessarily a reflection of inadequate host resistance. Since the fungus can evidently be isolated from the petioles of

a high percentage of the resistant plants, however, they obviously only tolerate the infection. This also means that under certain conditions tolerance may give way to susceptibility, and the planting may fail.

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EFFECT OF VARIATIONS IN TEMPERATURE ON THE REACTION OF TEMPERATURE-SENSITIVE WHEAT VARIETIES TO WHEAT STEM RUST

K. R. Bromfield

U.S. Army Chemical Corps Biological Laboratories, Fort Detrick, Maryland
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SUMMARY

The temperature-sensitive wheat varieties Kenya N.B. 263 and Lerma 52 are resistant to wheat stem rust races, 17, 38, and 56 at 70°F. Infected plants held as long as 18 days at 70°F and then transferred to 77° or 85°F did not retain the resistance. Sporulation typical for mycelium growing in susceptible tissue subsequently developed on the plants at the higher temperatures.

Kenya N.B. 263 and Lerma 52 are susceptible to races 17, 38, and 56 at 77° and 85°F. Infected plants held as long as 18 days at 77° or 85°F and then transferred to 70°F did not retain the susceptibility. Macroscopic evidence of incompatibility between host and fungus became apparent within 2 or 3 days after transfer to the low temperature with the formation of a ring of necrotic tissue surrounding each of the previously susceptible-type pustules. The ring of dead tissue did not develop immediately adjacent to the zone of sporulation, but was separated from it by a band of green tissue.

Introduction.—The reaction of most wheat varieties to many races of stem rust of wheat [*Puccinia graminis* Pers. var. *tritici* (Eriks. & Henn.) Shear, Ball, Jacks. & Stakman] is essentially constant over a fairly wide temperature range. There are wheats, however, whose reaction to stem rust is altered markedly by relatively slight temperature differences (2). Thus, certain varieties highly resistant at a given temperature become completely susceptible at a higher temperature. These varieties may be termed "temperature-sensitive."

It is important to know whether the resistance present in temperature-sensitive varieties at low temperature, or the susceptibility present at high temperature, is retained when these varieties are subsequently exposed to high or low temperature. The following tests were made to study this.

Materials and Methods.—Temperature-sensitive varieties Kenya N.B. 263, J(L), C 9968, Cal. 3098 (P.I. 117526), and Lerma 52, 461-6L-4L-2L-1I, and stem rust races 17, 38, and 56 were used.

The procedures followed in culturing and handling inoculum and in growing, inoculating, and incubating test seedlings have been described in a previous paper (2).

Observations were made daily on all inoculated plants for a period of at least 22 days after inoculation.

Results.—*Transfers of infected plants from a low temperature to high temperatures.*—Plants of Kenya N.B. 263 and Lerma 52 inoculated with rust races 17, 38, and 56 were placed at 70°F upon removal from dew chambers. At regular intervals of 1, 2, or 3 days thereafter, pots were removed from this temperature, placed in greenhouses controlled at 77° and 85°F, and held for the duration of the experiment. In all experiments, check plants were held continuously at each of the 3 temperatures.

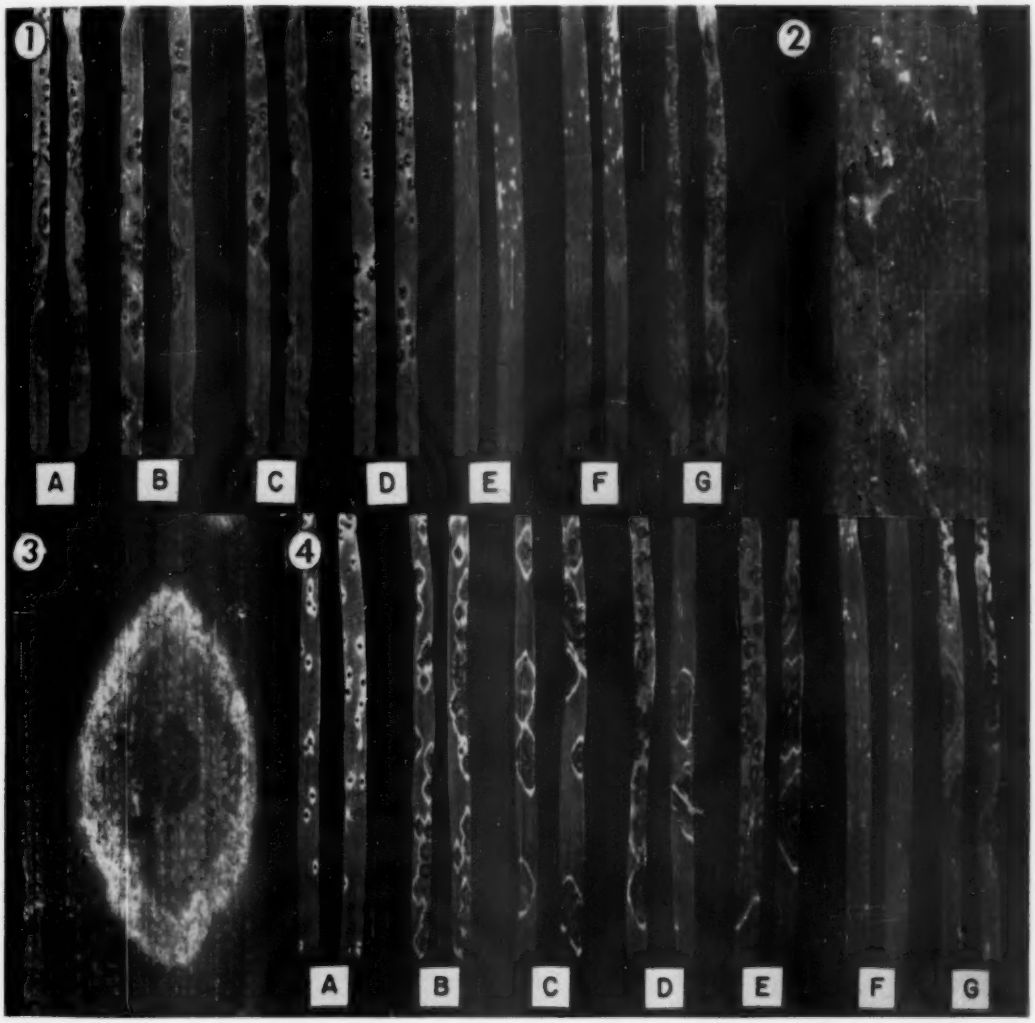

Fig. 1–4. Kenya N.B. 263 infected with race 38. **1)** Leaves A, B, C, D, and E were transferred from 70° to 85°F at 3, 6, 9, 12, and 15 days after inoculation, respectively. F and G are checks kept at 70° and 85°F, respectively. Photograph taken 22 days after inoculation. **2)** Primary and secondary pustule around necrotic fleck. Leaf held at 70°F for 9 days, then transferred to 85°F and held for 13 days. Only infection type 0; present at time of transfer. (*ca.* ×6.) **3)** Necrotic ring around pustule. Leaf held at 77°F for 8 days, then transferred to 70°F and held for 7 days. Infection type 3—at time of transfer. Necrotic ring developed within 3 days at the lower temperature. (*ca.* × 10.) **4)** Leaves A, B, C, D, and E were transferred from 85° to 75°F at 3, 6, 9, 12, and 15 days after inoculation, respectively. F and G and checks kept at 70° and 85°F, respectively.

In view of the similarity of results obtained with both varieties and each of the 3 rust races at both 77° and 85°F, a discussion of any race-host combination can be considered representative for all. Table 1 summarizes results obtained with Kenya N.B. 263 infected with race 38 and transferred from 70° to 85°F at 3-day intervals.

Rust mycelium in infected plants held for 18 days or less at 70°F subsequently sporulated when transferred to temperatures of 77° or 85°F. In contrast, mycelium in plants held for 26 days at 70°F, prior to transfer to 77° or 85°F, did not sporulate at the higher temperatures. Apparently, the rust mycelium remained alive in or near the necrotic flecks (which developed at low temperature) for 18 days at 70°F, but did not survive for 26 days at this temperature.

Rust on plants transferred from 70° to 77°F followed the same sequence of development as on

TABLE 1. Summary of treatments and observations of rust development on Kenya N.B. 263 inoculated with race 38 and held at 70°F for 3, 6, 9, 12, and 15 days and then transferred to and held at 85°F

No. of days at 70°F	Infection type at time of transfer from 70°–85°F	No. of days at 85°F	Rust development 22 days after inoculation
3	0	19	Infection types 3 and 4.
6	0;	16	Infection types 3 and 4.
9	0;	13	Infection type 3. Necrotic areas visible in the center of some pustules.
12	0;	10	Necrotic flecks surrounded by sporulation.
15	0;	7	Infection types 0; to 1.
22		0	Infection type 0;.
0		22	Infection type 4.

plants transferred to 85°F, but the time required to reach comparable susceptible-type sporulation was greater at 77°F. The ultimate infection type at 77° and 85°F was similar, however (Fig. 1, 2).

On the basis of these experiments, it was concluded that the resistance of temperature-sensitive varieties at low temperature is not retained when the infected plants are subsequently exposed to a high temperature.

Transfers of infected plants from high to low temperatures.—Seedlings of Kenya N.B. 263 and Lerma 52 inoculated with races 17, 38, and 56 were placed in greenhouses controlled at 77° and 85°F immediately upon removal from dew chambers. At 1-, 2-, or 3-day intervals thereafter, seedlings were transferred from 77° and 85°F to 70°F. They were held at the low temperature for the duration of the experiment.

Results obtained with Kenya N.B. 263 infected with race 38 (Table 2) are typical of those obtained with the other race-host combinations.

Host and parasite were compatible at 77° and 85°F. Upon transfer to 70°F, however, compatibility was lost, as evidenced by the development of necrotic tissue surrounding the rust pustules. Plants held not longer than 4 days at 77°F, or 3 days at 85°F, prior to transfer to 70°F, produced infection types 1 and 1+. On plants held longer than this at 77° or 85°F prior to transfer to 70°F, a very pronounced ring of necrotic tissue developed around each pustule within 3 days after transfer. The necrotic tissue did not develop adjacent to the sporulation but was separated from it by an annulus of green, normal-appearing tissue. Sporulating areas were the size of normal susceptible-type pustules. The final appearance resembled that of the classical "green island" of infection type 2, but with a much larger sporulating area (Fig. 3, 4).

Histological examination of cleared and stained leaves (1) showed that the necrotic ring coincided with the outer edge of the mycelial mat associated with the pustule. No rust mycelium was detected beyond the outer boundary of the necrotic ring. The host tissue between necrotic ring and pustule remained green and apparently healthy, although this region contained rust hyphae which had ramified through it when the plant was at the high temperature.

TABLE 2. Summary of treatments and observations of rust development on Kenya N.B. 623 inoculated with race 38 and held at 85°F for 3, 6, 9, 12, and 15 days and then transferred to and held at 70°F

No. of days at 85°F	Infection type at time of transfer from 85°–70°F	No. of days at 70°F	Rust development 22 days after inoculation
3	0;	19	Small pustules surrounded by a narrow band of chlorotic host tissue.
6	3–	16	Small pustules in "green islands" each surrounded by a narrow, sharply delineated ring of necrotic host tissue.
9	4	13	Large pustules in "green islands," each surrounded by a ring of necrotic host tissue.
12	4	10	Large pustules in "green islands," each surrounded by a ring of necrotic host tissue.
15	4	7	Large pustules in "green islands," each surrounded by a ring of necrotic host tissue.
0		22	Infection type 0;.
22		0	Infection type 4.

The evidence of incompatibility between host and fungus at low temperature appeared only at the outer boundary of the mycelial mat. In this region, the hyphal tips of the fungus may be in a more active metabolic state than the older hyphae in the region closer to the center of the mat. The reaction between host and fungus, expressed as death of host tissue and curtailment of fungus growth, may occur where this high state of activity exists.

On the basis of these experiments, it was concluded that susceptibility at high temperature is not retained when temperature-sensitive varieties are subsequently exposed to a sufficiently low temperature.

DISCUSSION.—For a temperature-sensitive variety, the standard reaction type at any given time cannot be taken as an indication of the "resistance" or "susceptibility" of the variety when this variety is exposed to varying temperatures which exceed the limits beyond which it would be "resistant" or "susceptible" if kept always at one or the other temperature. At one temperature, the host and fungus are compatible; at another, they are not. When such a host is subjected to changing temperature, the macroscopic appearance of resulting pustules is greatly influenced by the value of the temperature and length of exposure at a given temperature.

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PHYTOPATHOLOGICAL NOTES

A METHOD OF MAINTAINING VIABILITY AND ABILITY TO SPORULATE IN ISOLATES OF ASCOCHYTA

L. H. Person

Department of Plant Pathology, North Carolina State College, Raleigh.

Plant Pathologist, Crops Research Division, ARS, U.S.D.A., Raleigh, North Carolina.

A number of isolates of *Ascochyta* were obtained from 16 different hosts for a comparative study of pathogenicity on different hosts. Difficulty was experienced in obtaining sporulation and maintaining sporulating ability of many isolates. Kelman (4) reported that virulent cultures of *Pseudomonas solanacearum* E. F. Sm., could be maintained in sterile distilled or tap water at 21°C. Cultures were viable after 220 days at 21°C, and the percentage of cultural variants was less than 1. Kelman and Person (5) reported that such cultures of *P. solanacearum* were still viable and pathogenic after storage at 22°C for 18–24 months. In a comprehensive literature review by Fennell (2) on conservation of fungus cultures, no mention was made of maintenance of fungus cultures by the water-storage method.

The purpose of the present investigation was to determine whether viability and virulence of species of *Ascochyta* could be maintained by storage in water, as previously reported for *P. solanacearum*, and whether cultures would sporulate after transfer to solid media.

The following species were used: *Ascochyta melonis* Poteb. (*Mycosphaerella melonis* (Pass.) Chiu & J. C. Walker), 5 isolates obtained from cantaloupe, cucumber, and watermelon; *A. chrysanthemi* F. L. Stev. (*Mycosphaerella ligulicola* K. F. Baker, Dimock, & L. H. Davis), 1 isolate from chrysanthemum; *Ascochyta* spp., 33 isolates obtained from rhubarb, gourd, squash, watermelon, cantaloupe, cucumber, and pumpkin; *A. phaseolorum* Sacc., 15 isolates obtained from okra, snap bean, lima bean, cowpea, pepper, tobacco, cotton, and eggplant. Crossan (1) proposed that 7 *Ascochyta* spp. be reduced to synonomy with *A. phaseolorum* Sacc. which has priority in nomenclature.

Oatmeal-dextrose agar plates were streaked with aqueous spore suspensions and incubated at 24°C. Within 7–10 days, moderate to abundant sporulation of most isolates occurred; however, some isolates sporulated less. Approximately 10 ml of distilled water were placed in test tubes and sterilized. The tubes then were placed in controlled temperature storage compartments for several hours to attain the desired temperatures at which the isolates were stored. Transfers of 6 sporulating isolates were made in January, 1959, by placing an agar block approximately 0.75 x 1.5 cm in a test tube of sterile distilled water. These tubes were immediately placed at 1°C for storage. Similar transfers were made of 22 isolates in March and of 26 isolates in April, 1959; these were immediately placed in storage at 10–12°C. After 20–25 months, viability of all isolates was determined by agitating the tubes and streaking 2 loops of a spore suspension on petri dishes of oatmeal-dextrose agar. All isolates were viable and sporulated in 7–10 days with no apparent loss of ability to sporulate. This is a simple method for maintaining viability and sporulation of several *Ascochyta* spp. for 20–25 months.

In addition, 1 isolate of the *Colletotrichum* sp. of Jenkins and Winstead (3) has been maintained by this method and sporulation was abundant after 6 months.

Four isolates of *A. phaseolorum*, 1 each from okra, cotton, tobacco, and lima bean, were tested for pathogenicity after being stored 20–25 months in water. Spore suspensions of the isolates were streaked on oatmeal-dextrose agar plates and all isolates were sporulating abundantly in 10 days. Cotton and tobacco plants were inoculated by atomizing with a spore suspension of 10-day-old cultures. The plants were incubated in a moist chamber at 65°F for 60 hours. Each host showed typical *Ascochyta* leaf spot symptoms 12 days after inoculation with the 4 isolates tested. In addition to viability and sporulating ability, virulence of isolates of *Ascochyta* also was maintained after 20–25 months of storage in water at temperatures of 1° and 10–12°C.

The water-storage method may have application for maintaining viability, sporulation, and virulence of other pycnidium producing fungi, or for other fungi in which sporulation is maintained on solid media with difficulty.

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USE OF 1,2-DIBROMO-3-CHLOROPROPANE AS A FUNGICIDE AGAINST PYTHIUM ULTIMUM

Bill B. Brodie

North Carolina State College, Raleigh.

Plant Pathologist, Crops Research Division, ARS, U.S.D.A., Raleigh, North Carolina.

The effectiveness of 1,2-dibromo-3-chloropropane (DBCP) in controlling certain plant parasitic nematodes is well known. Investigations have shown that DBCP is effective as either a row application or seed treatment in controlling sting nematode, *Belonolaimus longicaudatus* Rau (1); and root-knot nematode, *Meloidogyne incognita acrita* Chitwood (3, 4) of cotton. In addition to increasing plant vigor, treatment of soil with DBCP usually results in increased plant stands (4). Increased stands are generally attributed to a reduction in the plant parasitic nematode population which in turn reduces fungus-nematode disease complexes (4). No reports which attribute an increase in plant survival to fungicidal properties of DBCP have been found. A closely related compound, 1-chloro-3-bromopropene-1, however, is reported fungicidal to *Pythium ultimum* Trow and other soil fungi (2). The failure of *P. ultimum* to attack cotton seedlings grown in DBCP-treated soil heavily infested with the fungus suggests fungicidal action. This investigation is concerned with the possible fungicidal action of DBCP *in vitro* against *P. ultimum* and the effectiveness of DBCP as a soil treatment to control damping-off of cotton seedlings caused by this pathogen.

Materials and Methods.—A virulent isolate of *P. ultimum* was maintained on sterile sorghum grain. In laboratory tests, *P. ultimum* was grown in the presence of different concentrations of DBCP (70.7% emulsible concentrate). Various dosages of DBCP were prepared by diluting the emulsible concentrate with distilled water. Two sterile analytical filter discs (12.7 mm) were soaked for 60 seconds in the desired concentration and placed on the surface of potato-dextrose agar in a 9-cm petri plate with 5 cm of agar surface between the discs. DBCP vaporizes into the atmosphere of the petri dish, but not into the agar medium. One sorghum grain infested with *P. ultimum* was placed in the center of the plate. The plates were held at 26°C and the diameter of the fungus colony was measured after 72 hours.

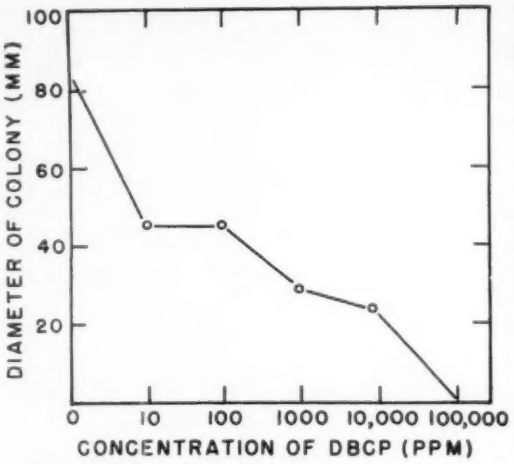

Fig. 1. Influence of various concentrations of DBCP on the growth of *Pythium ultimum* on potato-dextrose agar.

Soil treatment tests were made in the greenhouse. Cotton seedlings were grown in soil artificially infested with *P. ultimum* and treated with DBCP (17.3% granular). Six-in. pots were filled with sandy loam which had been treated with methyl bromide 1 month earlier. The potted soil was inoculated with *P. ultimum* by placing 5 infested sorghum grains in each pot of soil and watering daily for 1 week. DBCP was added to the pots 4 in. below the soil surface at rates of 1.0 and 1.5 gal/acre. Five days after treatment, 10 Coker 100W cotton seeds were planted in each pot. When the seedlings began to emerge, the temperature was held at 55°F for 4 days to enhance disease development. During the remainder of the experiment, the

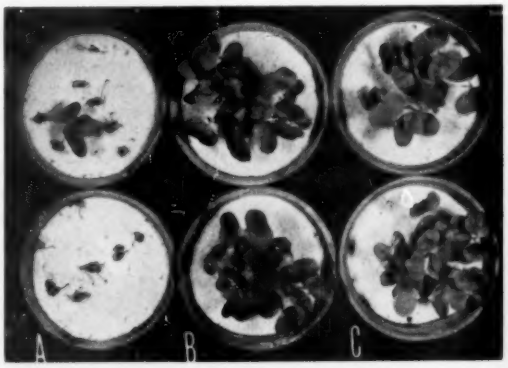

Fig. 2. Influence of soil treatment with DBCP on survival of cotton seedlings grown in soil: **A)** infested with *Pythium ultimum*, **B)** infested with *P. ultimum* and treated with DBCP (1.5 gal/acre), and **C)** sterile soil.

TABLE 1. Influence of DBCP on the survival of cotton seedlings grown in soil artificially infested with *Pythium ultimum*

Treatment		
Fungus	Chemical	% surviving seedlings
None	None	100.0
None	DBCP (1.0 gal/acre)	100.0
None	DBCP (1.5 gal/acre)	100.0
P. ultimum	DBCP (1.0 gal/acre)	94.6
P. ultimum	DBCP (1.5 gal/acre)	98.7
P. ultimum	None	11.3

seedlings were kept at 75–85°F. The number of surviving seedlings was recorded 2 weeks after planting.

RESULTS AND DISCUSSION.—Results of the present experiments indicate that DBCP is fungicidal to *P. ultimum*. In laboratory tests, 10 ppm DBCP partially inhibited the growth of *P. ultimum*. Complete inhibition of *P. ultimum* was obtained with 100,000 ppm DBCP (Fig. 1). Inoculum transferred from treated plates to nontreated plates indicated that DBCP was lethal at 100,000 ppm.

Excellent control of cotton seedling damping-off was obtained from soil treatment with DBCP at 1.0 and 1.5 gal/acre (Table 1). There were no visible phytotoxic effects (Fig. 2). Higher concentrations of DBCP were required to inhibit fungus growth completely than were needed to increase seedling survival. A more rapid escape of DBCP from the petri dish or differences in substrates could account for the lack of correlation of the results obtained by the 2 test methods.

The present study indicates that where both plant parasitic nematodes and *P. ultimum* are present in the soil, some of the benefits gained from soil treatment with DBCP are the direct result of damping-off control. DBCP is fungicidal at rates commonly used for nematode control, suggesting its usefulness in soils infested with both pathogens.

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STERILE CULTURE TECHNIQUES AS TOOLS IN PLANT NEMATOLOGY RESEARCH

Tseh-an Chen, R. A. Kilpatrick, and Avery E. Rich

Respectively, Research Analyst, New Hampshire Agricultural Experiment Station; Plant Pathologist, Crops Research Division, ARS, U.S.D.A.; and Plant Pathologist, New Hampshire Agricultural Experiment Station.

Cooperative investigations of the Crops Research Division, ARS, U.S.D.A.; and Department of Botany, New Hampshire Agricultural Experimental Station, Durham.

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Clover seedlings have been grown on filter paper in sterile water in test tubes and have been inoculated with various fungi as a test of pathogenicity of the fungi (1, 2). Chen (unpublished data) has modified this technique by using inorganic nutrient agar in place of sterile distilled water, permitting the plants to survive for several months. This method is advantageous in conserving space, plants, and inoculum. There is little risk of contamination. The roots can be examined at any time, permitting observation of the progress of pathogens attacking the roots of a susceptible host. This technique has been further modified and should prove a useful tool in plant nematology research.

Ladino white clover (*Trifolium repens* L.) seeds were scarified for 10 minutes in concentrated sulfuric

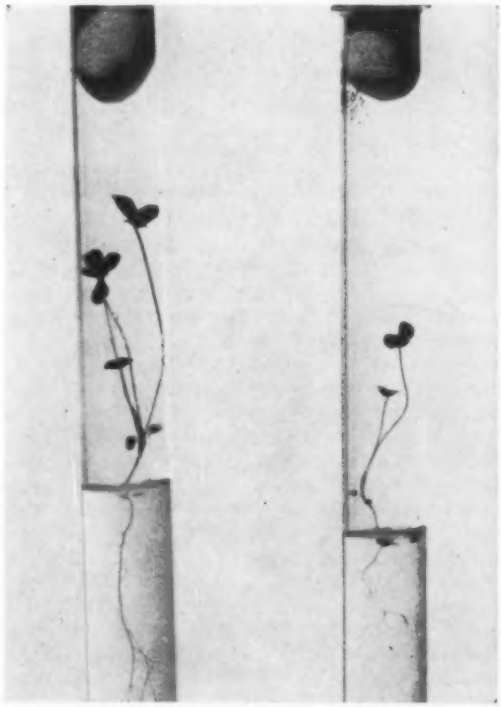

Fig. 1. Test tubes showing growth of white clover seedlings under aseptic conditions. Uninoculated control, left; nematode-infested tube, right. Nematodes were introduced 12 days after seedlings were placed in the tubes; the photograph was taken 24 days later.

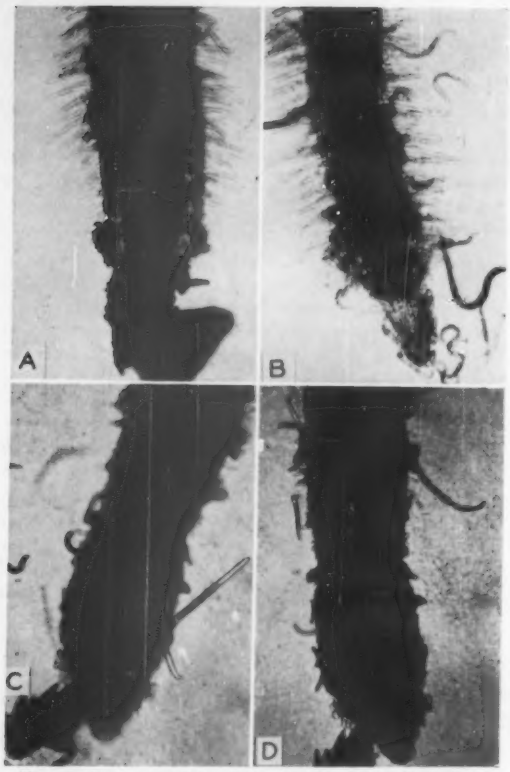

Fig. 2. Roots of white clover photographed through test tubes (**Fig. 1**) showing stages of injury caused by *Pratylenchus penetrans*: **A)** uninoculated control; **B)** early stage of feeding; **C, D)** late stages showing severe damage to root hairs. Approximately × 500.

acid, washed in sterile distilled water, germinated in a sterile petri dish on moist filter paper, and transferred aseptically to test tubes (150 x 18 mm) containing a sterile medium. The medium used was a modified Hoagland's and Knop's solution, prepared as follows and adjusted to a pH of 6.0: macroelements, Ca $(NO_3)_2 \cdot 4H_2O$, 0.95 g, KNO_3, 0.61 g, $MgSO_4 \cdot 7H_2O$, 0.49 g, $NH_4H_2PO_4$, 0.12 g; microelements (stock solution, 1,000 ml in vol), $MnSO_4 \cdot 4H_2O$, 3.0 g, $ZnSO_4 \cdot 7H_2O$, 0.5 g, H_3BO_3, 0.5 g, $CUSO_4 \cdot 5H_2O$, 0.025 g, $Na_2MoO_4 \cdot 2H_2O$, 0.025 g, H_2SO_4 (sp gr 1.83), 0.5 ml; ferric citrate (stock solution, 1,000 ml in vol), $FeC_6O_5H_7 \cdot 5H_2O$, 10 g, agar, 5.0 g, distilled water, to 1,000 ml.

One ml of the microelement-stock solution and 2 ml of the ferric citrate stock solution were used for each liter of medium.

The above medium provided excellent growth of host plants tested. To prevent dehydration of the medium and plant, each tube was plugged with cotton and covered with a piece of polyethylene held in place by a rubber band. Then, the seedlings were grown under a fluorescent light containing two 40-w daylight-type lamps (Fig 1). A single species-culture of *Pratylenchus penetrans* (Cobb) Sher & Allen was obtained from Ladino white clover. The nematodes were disinfested by immersion in a solution of streptomycin sulfate (0.1%) and malachite green (30 ppm). Then, they were transferred to sterile corn roots growing in tissue culture on a modified White's medium (3), where they multiplied. Sections of corn root containing approximately 15–20 nematodes were transferred to test tubes containing clover seedlings growing on the artificial medium.

The movements and progress of the nematodes were followed by direct, microscopic observation. Within 24 hours, they moved downward into the agar toward the tip of the longest root, before starting to feed on the root hairs and root tip just back of the root cap (Fig. 2-B).

Uninoculated plants produced white healthy roots with an abundance of root hairs (Fig. 2-A). Roots of inoculated plants, on the other hand, had very few root hairs (Fig. 2-C, D). The nematodes concentrated at the root tip and fed in that area. Some were observed inside the roots. After the root tips turned brown, the nematodes migrated to nearby branch roots and attacked these root tips. Thus, it was ascertained that nematodes alone can cause considerable damage to roots in the absence of fungi. The number of root hairs was considerably reduced, the growing point or root tip was badly damaged, and wounds were produced through which fungi could easily gain entrance. Parasitized plants became pale, stunted, and showed a general lack of vigor.

The above method provides a pure-culture technique for studying actual damage to roots caused by nematodes alone. The effect on aerial symptoms, as well as roots, can be observed at periodic intervals without disturbing the plants or the nematodes.

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INOCULATION TECHNIQUE FOR DETERMINING TOLERANCE OF PISUM SATIVUM TO APHANOMYCES EUTEICHES

W. A. Haglund and T. H. King

Department of Plant Pathology and Botany, University of Minnesota, St. Paul.

Respectively, former Research Assistant and Professor.

Paper No. 4631, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

Several methods have been used to determine the tolerance of peas to common root rot, caused by *Aphanomyces euteiches* Drechs. One method, developed by Johnson and Bissonnette (unpublished, University of Minnesota), consisted of inoculating soil in which peas were growing with a known concentration of zoospores of *A. euteiches*. The method was effective for screening varieties and plant introduction collections for tolerance to common root rot; it was not sensitive enough, however, to detect small differences in tolerance to common root rot. A technique developed by W. T. Schroeder (personal communication), Geneva, N.Y., consisted of growing pea seedlings in a sand hydroponic system. At the desired age of the peas, the sand was saturated with water containing zoospores of *A. euteiches*. Schroeder's method required special equipment which was not available to us. In addition, the pea seedlings he had were subject to attack by the organism only during a limited period of development. Therefore, a method of inoculation was developed that required a minimum of equipment and was reliable in the evaluation of peas resistant or tolerant to *A. euteiches*.

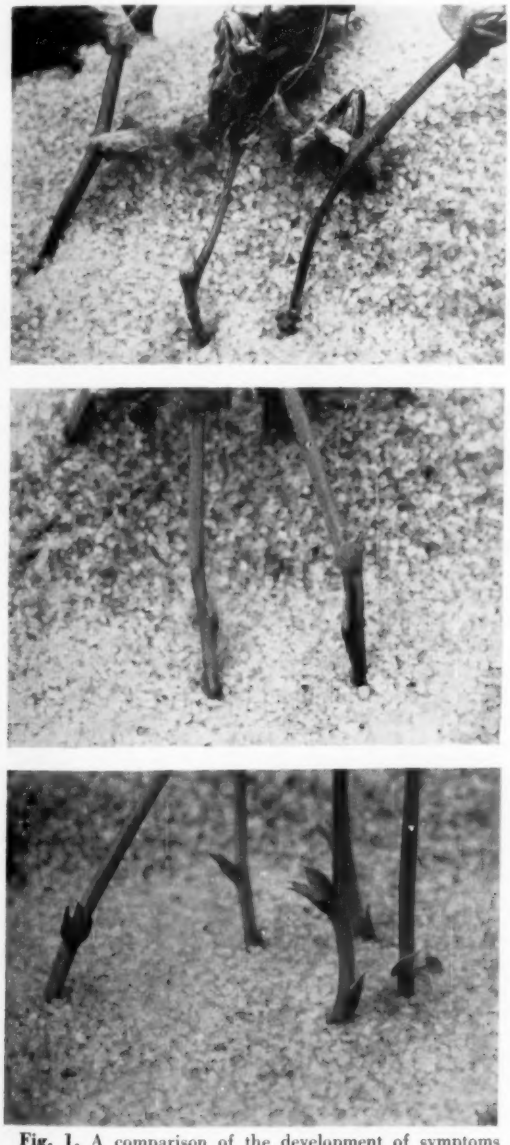

Fig. 1. A comparison of the development of symptoms on 3 varieties of peas inoculated with 100,000 zoospores/plant of isolate 2 of *Aphanomyces euteiches*. Top, New Era (susceptible); middle, Alaska (tolerant); bottom, Minnesota 494A-1 (resistant).

TABLE 1. Percentage of pea seedlings killed when inoculated with varying concentrations of zoospores from *Aphanomyces euteiches*

Pea selections	Zoospore concentration/plant (thousands)	Isolate 2 (%)	Isolate 4 (%)	Isolate 10 (%)
New Era	25	100	47	80
	50	100	20	87
	100	100	40	100
Alaska	25	26	13	40
	50	26	54	54
	100	40	60	47
Minnesota 494A-1	25	0	0	0
	50	0	0	0
	100	0	0	0

METHODS.—The fungus was grown on a corn water medium for 7 days at 20°C. The mycelial mats were removed, washed in tap water, and incubated at 20°C for 24 hours. A haemocytometer was used to determine the desired concentration of zoospores, which were added to 10 ml of water as inoculum.

Pea seeds were germinated in trays of autoclaved Vermiculite. Seedlings were grown 7 days at 21°C, removed, and the root systems washed in tap water. The plants were suspended in a shallow plastic tray containing an aqueous suspension of *A. euteiches* zoospores. The plants were incubated at room temperature for 24 hours, removed from the inoculum, and transplanted into 5-in. clay pots containing steamed soil.

Three single-spore cultures of *A. euteiches* were used. Cultures 2 and 4 were originally isolated from diseased peas grown in Minnesota and culture 10 was made available by W. T. Schroeder. Three concentrations of zoospores, 25,000, 50,000, and 100,000/plant, were used to inoculate 3 varieties or lines of peas. The New Era and Alaska varieties and Minnesota selection 494A-1 were selected because results of previous studies had indicated that New Era was susceptible; Alaska, tolerant or moderately resistant; and Minnesota 494A-1, the most resistant selection.

RESULTS.—Table 1 presents the data obtained on the number of seedlings killed by the 3 isolates on the 3 varieties and selections of peas. Culture 2 was the most pathogenic and killed the greatest percentage of

seedlings. Culture 10 was moderately pathogenic and culture 4 the least pathogenic.

Fig. 1 illustrates the difference in the severity of symptoms on the 3 varieties inoculated with culture 2 at a concentration of 100,000 zoospores/plant. The effect on the New Era seedlings occurred at an early stage of development; that on the Alaska variety was much later in relation to plant development.

DISCUSSION.—The uniformity and effectiveness of this method of inoculation makes it possible to evaluate the tolerance of a variety to common root rot. A uniform infection and destruction of roots of seedlings was obtained with the pathogenic isolates of *A. euteiches*. With the weakly pathogenic isolates of *Aphanomyces*, however, the results were not so consistent. The method effectively screens varieties or lines of peas for tolerance to this disease and determines the relative pathogenicity of various isolates of the fungus. In addition, with this method, tolerant seedlings can be maintained in pots in the greenhouse until seed is produced; no special equipment is needed.

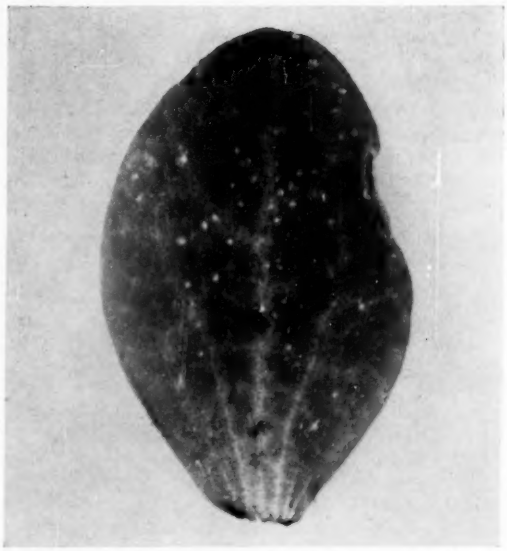

Fig. 1. Local lesions induced by TRSV on squash cotyledons.

SEPARATION AND IDENTIFICATION OF SOME VIRUSES INFECTIOUS TO CUCURBITS

R. E. Webb

Plant Industry Station, Beltsville, Maryland.
Plant Pathologist, Crops Research Division, ARS, U.S.D.A., Beltsville.

Cucurbit viruses are widely distributed over the United States (1, 2, 3, 4, 6); and frequently, infected plants contain 2 or more viruses (3, 5, 8). Diagnostic hosts and methods are known for separating and identifying cucumber mosaic virus (CMV), squash mosaic virus (SMV), and watermelon virus (WMV) in mixtures (3, 4). Tobacco ringspot virus (TRSV), which is widespread throughout the cucurbit-growing areas of the Central United States, (5, 6, 7) frequently occurs in mixed infection with CMV, SMV, and WMV (5, 8). TRSV has a wide host range and a rapid method of separating in pure culture other viruses infecting cucurbits from mixtures with TRSV would greatly facilitate virus identification.

Cucurbit specimens containing TRSV alone and in combination with CMV, SMV, and WMV were received from southern Texas during June and October, 1960. All viruses were easily transmitted to various cucurbits and other diagnostic hosts during both periods. Contrary to reports (5, 6), TRSV was easily recovered from infected specimens of Charleston Gray watermelon, a diagnostic host for WMV. Also, it was infectious to hosts of SMV.

During routine inoculation tests, when TRSV was inoculated on cotyledons of Early Prolific Straightneck squash, the virus induced small, grayish-white local necrotic lesions (Fig. 1); but, in many instances, the virus did not become systemic. Studies were initiated to determine the efficiency of inoculation of squash cotyledons in separating CMV, SMV, and WMV in pure culture from TRSV.

Ten squash plants were placed at each of 4 temperatures and inoculated in the cotyledon stage with inocula from squash infected with both TRSV and WMV. The influence of temperature on the systemic invasion of the plants with both viruses was studied. At each temperature, 10 plants in the 2-true leaf stage were inoculated with a mixture of viruses and served as controls. Local lesions developed on the squash cotyledons at all 4 temperatures. TRSV became systemic only at 28°C and in only 4 of 10 plants (Table 1). Plants held at each temperature developed systemic symptoms of WMV. Plants held at 16°C for 3 weeks were moved to 20° for symptom development.

TABLE 1. Influence of temperature on separation of TRSV from WMV by inoculating cotyledons of squash

Temperature (°C)	Plant growth stage	Plants systemically infected with:[a] TRSV	WMV
16	Cotyledon	0	10
	True leaf	10	10
20	Cotyledon	0	10
	True leaf	10	10
24	Cotyledon	0	10
	True leaf	10	10
28	Cotyledon	4	10
	True leaf	10	10

[a] Of 10 inoculated.

TABLE 2. Separation of tobacco ringspot virus (TRSV) from mixtures with other cucurbit viruses by cotyledon inoculation of squash

Virus or virus mixture inoculated on squash	Squash plants systemically infected with TRSV (of 12 inoculated)	Predominant virus symptom
TRSV (leaf inoculation)	12	TRSV
TRSV	0	None
CMV (cucumber mosaic virus)	0	CMV
SMV (squash mosaic virus)	0	SMV
WMV (watermelon mosaic virus)	0	WMV
TRSV + CMV	0	CMV
TRSV + SMV	0	SMV
TRSV + WMV	0	WMV
TRSV + CMV + SMV	0	CMV
TRSV + CMV + WMV	0	CMV
TRSV + SMV + WMV	0	SMV

All control plants became systemically infected with both viruses.

Earlier (8), TRSV from hosts other than squash induced local lesions; but frequently did not become systemic when inoculated on squash cotyledons. Groups of 10 squash plants in the cotyledon stage were inoculated with TRSV from infected cantaloup, cucumber, squash, and watermelon to study a possible virus-source influence on the development of systemic symptoms in squash. Inoculum from all 4 hosts induced typical local lesions on the cotyledons, although inoculum from watermelon induced only 5–10 lesions/cotyledon. Systemic symptoms developed only in 4 plants inoculated from diseased squash and 6 plants from cantaloup.

The relative efficiency of cotyledonary separation of WMV, SMV, and CMV, from TRSV was studied. Twelve squash plants in the cotyledon stage were inoculated with mixtures of these viruses with TRSV from squash. TRSV did not become systemic, except in plants inoculated in the first 2-true leaf stage (Table 2). Each of the other viruses became systemic, as shown by subinoculations on diagnostic hosts. Abnormally cool greenhouse temperatures during the test period might have influenced these results. Similar tests with naturally infected cucurbit specimens have given similar results.

Reportedly, TRSV is difficult to transmit mechanically from watermelon foliage (5). Under the conditions of these studied, however, TRSV was transmitted easily and repeatedly from stems, leaves, small fruits, and floral parts of 3 specimens of Charleston Gray watermelon to several hosts.

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RADIATION AND TEMPERATURE INTERACTION ON THE SPORULATION OF ALTERNARIA TOMATO

Minoru Aragaki

Department of Plant Pathology, University of Hawaii, Honolulu.

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A general practice when studying environmental variables influencing physiological processes of fungi is to keep all but 1 factor constant. Reliable information has thus been obtained, but the possibility exists of overlooking significant interactions.

Witsch and Wagner (7) and Barnett and Lilly (1) demonstrated that sporulation of *Alternaria brassicae* var. *dauci* and *Choanephora cucurbitarum* (B. & Rav.) Thaxter, respectively, was divided into 2 physiological processes. Light was necessary for the initial process but was apparently inhibitory to the second. Barnett and Lilly, however, showed that with *C. cucurbitarum* both processes could take place under low light intensity.

In several *Sclerospora* spp., Weston (6) found that conidial production took place only at night or very early morning. Melhus, *et al.* (4) and Hiura (2) have observed sporulation of *S. graminicola* (Sacc.) Schroet. under daylight conditions in laboratory and field experiments, however, and indicated that low temperatures are required for sporulation.

Working with *A. solani* (Ell. & Martin) Jones & Grout, *A. passiflorae* Simmonds, *A. porri* (Ell.) Neerg., and *A. dianthicola* Neerg., it was observed, as others have (3, 5), that the large-spored isolates sporulated poorly; alternation of light and darkness seemed nec-

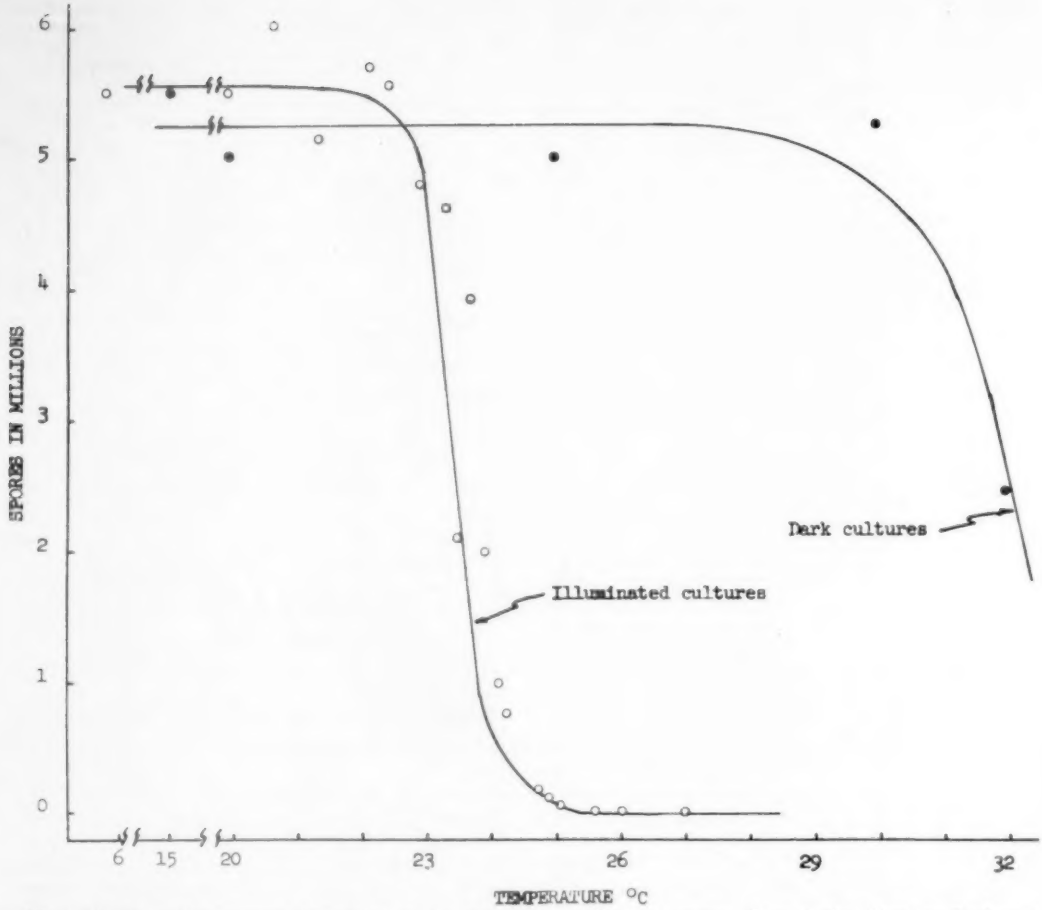

Fig. 1. Light and temperature interaction on the sporulation of *Alternaria tomato*. Open circles, illuminated cultures; filled circles, dark cultures.

essary for sporulation but low temperatures could be substituted for darkness.

MATERIALS AND METHODS.—An isolate of *A. tomato* (Cke.) Weber from *Passiflora edulis* Sims. was grown on a medium composed of 10% V-8 juice (a proprietary product), 0.2% $CaCO_3$, and 2% agar. Single spores were used to inoculate 40 ml of the medium in 250-ml Erlenmeyer flasks. The cultures then were grown continuously for 7 days at 26–31°C under a fluorescent lamp (General Electric Cool White F40 CW-3) with a light intensity of 300 ft-c at the level of cultures. During this period, no spores were formed.

After this initial growth period, duplicate cultures were immersed in a water bath for 22-hour treatments at temperatures between 6° and 27°C. The temperature of the water bath, in a walk-in refrigerator at 5°C, was regulated by a Merc-to-Merc Thermo-regulator; the sensitivity to the ambient temperature was ± 0.04°C, which was checked regularly with a Beckman differential thermometer. Cultures in the water bath were illuminated with a fluorescent lamp (Westinghouse Daylight 15W) at a light intensity of 100 ft-c at the culture surface. For dark induction of sporulation, duplicate cultures were placed in incubators having precision of ± 0.5°C.

After the 22-hour treatment, spore counts were made with a haemocytometer. When there were too few spores to count accurately with a haemocytometer, spore suspensions were placed in plastic chambers (46 mm x 46 mm) with grids and counts were made with a dissecting microscope.

RESULTS.—The number of spores of *A. tomato* produced under illumination at temperatures between 6° and 23°C, and in the dark between 15° and 30°C, was approximately 5×10^6/flask (Fig. 1).

A sudden drop in spore production occurred between 23° and 25°C in illuminated cultures. Above 25°C, virtually no spores were formed, demonstrating a high interaction between temperature and radiation in inhibiting sporulation of *A. tomato*.

A. solani, A. passiflorae, A. porri, A. dianthicola, Stemphylium botryosum Wallr., and *Helminthosporium turcicum* Pass. similarly did not sporulate under illumination at temperatures between 26° and 31°C; but they all sporulated profusely when transferred to illuminated incubators at 20°C or placed in the dark between 26° and 31°C. The sporulation of *Ascochyta pisi* Lib., *Pestalotia* sp., *Aspergillus* sp., and *Cunninghamella* sp. was not inhibited by light at temperatures up to 31°C.

DISCUSSION.—It is probable that the sporulation of many fungi is inhibited by light at high temperatures. Since these temperatures approximate laboratory temperatures, this may be a major consideration in obtaining fungus sporulation.

It would be interesting to see whether fungi which possess the mechanism inhibitory to sporulation would fall into certain taxa. Research is being continued along these lines.

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A TECHNIQUE FOR STUDYING RELATIVE SOIL POPULATIONS OF TWO FUSARIUM PATHOGENS OF GARDEN PEAS

Gayle L. Worf and D. J. Hagedorn

Department of Plant Pathology, University of Wisconsin, Madison.

Fusarium oxysporum Schlecht. f. *pisi* (Linford) Snyd. & Hans., race 2; and *F. solani* (Mont.) Appel & Wr. f. *pisi* (Jones) Snyd. & Hans., incitants of near-wilt and *Fusarium* root-rot of *Pisum sativum* L., respectively, are often isolated from roots of the same plants in Wisconsin. As a part of a study concerning the interactions of these 2 fungi, the details of which will be reported later, a technique was desired through which relative development of the 2 pathogens could be followed simultaneously in soil infested with both organisms. Since no simple and rapid method was available for satisfactorily accomplishing this, the following procedures were developed and used in the greenhouse with artificially infested soils.

TABLE 1. The soil population variation among replications of 4 treatments involving 2 species of *Fusarium* pathogenic to pea

Replication no.	Population level/ml, × 10^{-3} *			
	F. oxysporum f. *pisi* r. 2		*F. solani* f. *pisi*	
	Alone	With *F. solani*	Alone	With *F. oxysporum*
1	1,000	990	500	10
2	1,300	1,550	615	0
3	1,380	1,650	550	3
Average	1,227	1,363	555	4

*Each reading an average of 4 culture plate counts.

Sand-loam mixtures were seeded in different proportions and sequences with inocula of both fungi 1–3 weeks after sterilizing. On appropriate dates, sample cores of soil then were drawn using glass cylinders calibrated to hold 1 ml. In each crock to be sampled, such a sterilized cylinder was carefully forced into the soil, repeating as necessary until it was filled. Filling the tube generally required more than 1 probe; the first soil quantity was gently moved up the cylinder with a rod, care being taken not to pack the soil. After the cylinder was filled, the outside was cleaned of adhering soil particles and the soil forced out of the cylinder with the rod into 100 ml of sterile distilled water in a 250-cc Erlenmeyer flask. Three replications were made of each treatment. The flasks containing the water and soil samples were placed upon a rotary shaker for 15 minutes. Generally, 2 serial dilutions then were made from this suspension, making 3 final dilutions of $1{:}10^2$, $1{:}10^4$, and $1{:}5{\cdot}10^4$.

One-ml portions then were plated out from each of the 3 dilutions, 4 replications each, on petri plates containing potato-dextrose agar buffered at pH 3.1 with phosphate buffer and maintained at 20°C. This technique excluded growth of many fungi and all bacteria and also restricted colony sizes of both *Fusarium* species. At the same time, the colors distinguishing between *F. oxysporum* and *F. solani* colonies developed; and counts of each fungus could be made after 5–7 days. At pH levels below 3.0, *Fusarium* growth was frequently inhibited. At 12°C, growth of both species was scant and the pigment of *F. oxysporum* failed to develop. The fungi were not easily distinguishable at 16°; but at 20° *F. oxysporum* colonies were purple, whereas *F. solani* colonies were white with a more floccose growth (Fig. 1).

Growth of both fusaria could be initiated from either mycelial or conidial origin on this medium. The number of colonies increased with longer periods of shaking the soil suspension and results of different trials were comparable only if a standardized technique was followed.

Replications within treatments were averaged before treatment comparisons were made. Variation

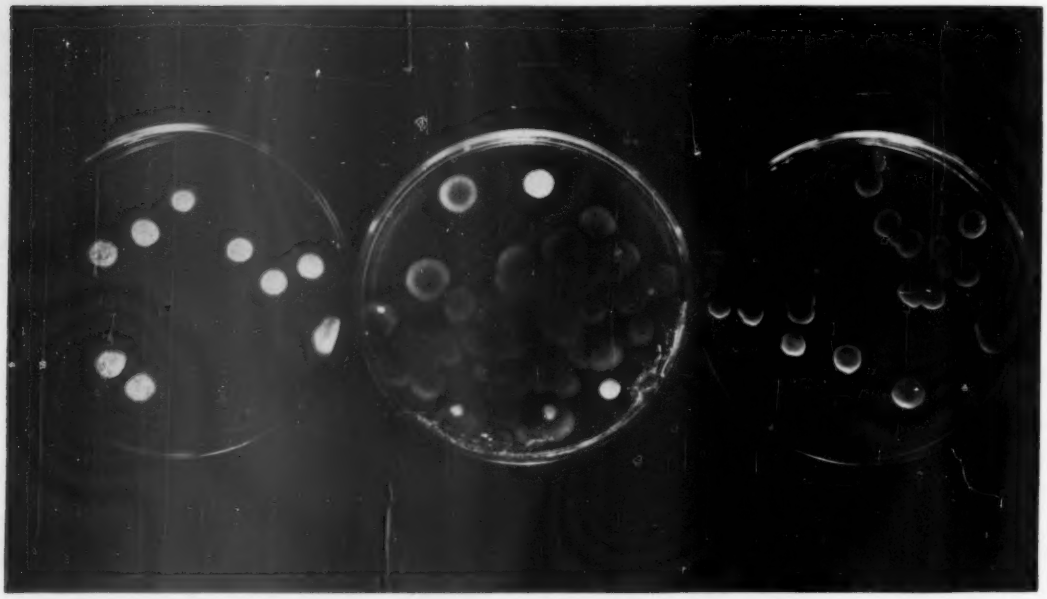

Fig. 1. Culture dishes of potato-dextrose agar, pH 3.1, inoculated with soil extracts, indicating how *Fusarium solani* f. *pisi* and *F. oxysporum* f. *pisi* race 2 can be distinguished. Left, white *F. solani* colonies from soil infested with the root rot fungus only; center, *F. oxysporum* and *F. solani* colonies from soil infested with both fungi; right, purple *F. oxysporum* colonies from soil to which only the near-wilt fungus had been introduced.

among replications was low considering that samples were drawn on a volume basis and the technique was sufficiently rapid to handle the required tests on a given day. Table 1 presents data illustrating the population variation among replications and treatments. No difficulty was encountered with sampling, providing the soil was light and not too wet. Excessive contaminants were a problem only in occasional trials. The greatest difficulty was encountered when population differences between the fungi were extreme, because the fungus present in the least numbers was not detectable at dilutions where counts of the more abundant species were possible. Even in such treatments, however, the trend of growth could be determined for each fungus.

TRICHODORUS CHRISTIEI AS A VECTOR OF POTATO CORKY RINGSPOT (TOBACCO RATTLE) VIRUS

C. H. Walkinshaw, G. D. Griffin, and R. H. Larson

Department of Plant Pathology, University of Wisconsin, Madison.

Respectively, Project Assistant, Department of Plant Pathology; and Nematologist and Plant Pathologist, Crops Research Division, ARS, U.S.D.A., Madison, Wis.

Soil-borne potato corky ringspot (tobacco rattle) virus was first reported from northern Florida in 1946 (2). The severity of the disease has varied from location to location as well as from year to year. Variability of environmental conditions could not satisfactorily explain the erratic occurrence of the disease (8).

The possibility of a nematode vector was considered because of the erratic occurrence of the disease under natural conditions and our inability to increase transmission by adding relatively large amounts of virus to steam-sterilized soil.

Virus-infested soil was obtained from A. H. Eddins of Hastings, Fla. In several of these samples, over 50% of the transplanted *Nicotiana tabacum* L. 'Samsun' plants became infected (7). These soils were used for nematode analysis. The soil was washed by Cobb's sifting and gravity method. The different plant parasitic nematodes, including *Trichodorus christiei* Allen, were hand-picked and the species separated. The nematodes were washed through several baths of distilled water. Approximately 30 of each species were added to steam-sterilized sandy loam in 30-ml glass beakers containing healthy Samsun tobacco plants. Supernatants from the nematode washings were added to a like number of plants for controls. The experiment was conducted in a greenhouse at 20 ± 2.5°C with a maximum day-length of 10 hours.

Tobacco plants growing in *T. christiei*-infested soil developed systemic symptoms of the virus (Fig. 1) in a minimum of 9 days. The transmission was confirmed

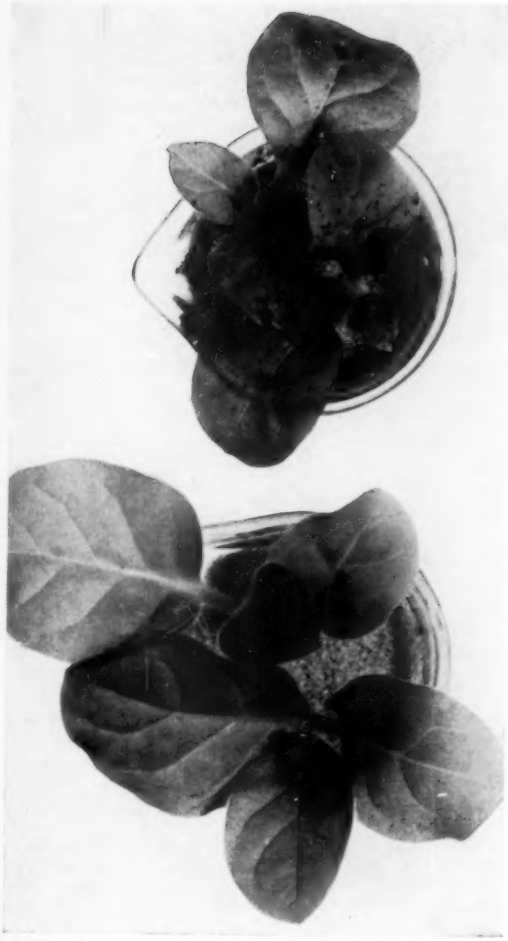

Fig. 1. Top, symptoms of tobacco rattle on Samsun tobacco grown in nematode-infested soil. Bottom, healthy tobacco grown in nematode-free soil.

by mechanical transmission of the virus from infected leaf tissue to differential hosts (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Phaseolus limensis* Macf., and *Vinca rosea* L.). Tobacco inoculated with other plant parasitic nematodes and with the supernatants from the nematode washings showed no disease symptoms.

Further studies were made to determine the numbers of nematodes needed to transmit the virus and the effect of different sizes of containers on the rate of virus transmission and incubation period.

Thirty-four-day-old seedlings of Samsun tobacco transplanted to sterile sandy loam (4-in. pots), to which different numbers of nematodes were added, became infected as follows: 10–15 nematodes, 58%; 20–25 nematodes, 83%; 30–40 nematodes, 83%; 50–150 nematodes, 92%. Each figure was based on 12 replications.

TABLE 1. Effect of corn rotation on incidence of tobacco rattle disease in different soil samples originally giving various percentages of infection of tobacco; each test involved 75-100 plants in metal pans 3 in. deep (8)

% infection of tobacco preceding corn	% infection of tobacco after corn	Days between transplanting and symptom appearance (avg.)	
		Before corn	After corn
0	0		
1	100	54	14
3	100		
12	100	23	15
18	100	16	16
24	100	32	31
28	100	21	17
40	100	20	15
86	100	21	26
96	100	19	25
98	100	20	17
100	100		

The containers used to check transmission of the virus ranged from 18-ml vials to 6-in. clay pots. In all cases, the minimum period between addition of nematodes and appearance of symptoms on tobacco plants was 8–10 days.

Successive plantings of Samsun tobacco in nematode- and virus-infested soil resulted in a decrease in the number of infected plants. This corresponds to a reduction in the population of the nematode vector since tobacco is a poor host of *T. christiei* (1) and since root necrosis is caused by the virus.

The incidence of infection increased when tobacco was rotated with corn (Table 1). This followed an increase in the nematode vector population, since corn is a good host of *T. christiei* (1) and since the virus failed to cause a necrosis of the corn roots. Also, virus multiplied in the corn roots as confirmed by mechanical transmission of the virus from these roots to T.I. 787 tobacco plants.

Previous studies (8) demonstrated certain consistent differences in potato corky ringspot virus types and suggested that these types might be closely related to strains of tobacco rattle virus reported from Europe (3, 4, 5). The "etching" isolate of potato corky ringspot virus completely protected Samsun tobacco plants from infection by all other strains of this virus found in Florida soils and from a variety of tobacco rattle virus cultures from European laboratories. These results and the previously reported (8) serological relationship between the potato corky ringspot virus of Florida and the European tobacco rattle virus indicate that the "etching" and "necrotic" strains of the potato corky ringspot virus are strains of tobacco rattle virus. Sol, *et al.* (6) also indicate the probability of tobacco rattle virus of Europe being nematode-transmitted, which tends to substantiate this relationship.

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CULTURAL VARIATION IN ENDOTHIA PARASITICA

J. O. Andes

Department of Plant Pathology, Agricultural Experiment Station, Knoxville, Tennessee.

The chestnut blight fungus [*Endothia parasitica* (Murr.) P. J. & H. W. Anderson] is generally characterized in culture on potato-dextrose agar by moderate growth rate, pigmentation from light yellow to tan, and abundant formation of conidia. Modification in growth rate, pigmentation, and pycnidial formation is brought about by kind of media, pH, moisture available, and other factors (2). Hence, variability based solely on the appearance of cultures is a poor criterion for judging biotypes. Nevertheless, in common with many fungi, variability was found among ascosporic isolates, which indicates genetic differences in an otherwise rather stable organism. These differences were observed when the separate ascospores from single asci were grown on a single petri dish. Variability was most pronounced when the cultures were about 10 days old; later, a more uniform appearance tended to develop.

Isolates from cankers obtained from chestnut sprouts in fall, 1960, were used for the study. Perithecia were crushed and spread on agar in petri dishes; spores were teased from the asci with glass needles under a stereoscopic microscope and allowed to germinate overnight. The complement of spores from each ascus then was transferred to another petri dish so that cultures could develop.

Growth rate of the colonies was about the same and the general appearance similar. At first, however, some were darker than others and tended to form heavier spore masses, both on potato-dextrose and corn meal agar media. After 1 week, some of the colonies merged, but others did not, showing a zone of inhibition 2-3 mm between colonies. In others, a definite ridge of pycnidia was formed. Various combinations then were made so that all ascosporic cultures from an ascus were mated with all others. One chestnut specimen yielded 7 complete sets and 14 with 7 spores each. A total of 247 matings obtained from the various sets showed ridges in 28.3% of the cases, an inhibition zone in 40.0%, and merging in 31.5%. Since the production of pycnidia in culture has been attributed to drying out of media (2), cultures were maintained on media of sufficient volume (40 ml/90 mm petri dish) and moisture to assure this was not a factor. No changes were observed after the first 10 days. Examination was made a month later.

No attempt is made to explain the behavior of these isolates, but it is suggestive of aversion as defined by Cayley (1), Voorhees (3), and others. Such reaction is indicative of physiological variation that is genetically controlled and could result in the production of strains of the organism. Possible differences in pathogenicity, however, must await inoculation tests. In no instance in the present case were perithecia found in the ridges between colonies; hence, the sexual situation cannot be postulated. Further examination of the problem is anticipated if perithecia develop on chestnut sprouts in the fall. Limited evidence at hand, however, suggests that the ascosporic stage of this fungus provides a mechanism for strain development.

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THE PIPE CLEANER, AN INEXPENSIVE TOOL FOR INOCULATING PLANTS WITH VIRUSES

K. W. Kreitlow

Plant Pathologist, Crops Research Division, ARS, U.S.D.A., Beltsville, Maryland.

Mechanically transmitted viruses are introduced into healthy host tissue by several commonly used techniques. The most popular methods include rubbing healthy leaves with a cheesecloth pad, glass spatula, brush, or forefinger dipped in virus inoculum. Abrasives may or may not be used. Each of the tools mentioned has advantages and limitations. One of the foremost disadvantages of most methods is the possibility of contamination, unless hands and implements used are thoroughly washed or disinfected between inoculations. Washing and disinfecting are time-consuming, particularly when several virus isolates are

being indexed on tester plants. To overcome some of the disadvantages of other methods, an inexpensive, disposable, readily available tool, the pipe cleaner, has been adapted to inoculate plants artificially with mechanically transmitted viruses.

Pipe cleaners can be bent into any desired shape. It has been most satisfactory to bend a pipe cleaner double, preferably around a pencil tip or similar small object to form a loop of the desired size. The ends of the pipe cleaner then are twisted to form a handle. The doubled pipe cleaner possesses sufficient stiffness to perform most rubbing operations. If the pipe cleaner loop is placed in the virus inoculum, enough inoculum usually is soaked up or held in the loop to inoculate a bean or a tobacco leaf adequately without repeated dipping into the inoculum. Carborundum powder or similar abrasives may be dusted on the leaf or suspended in the inoculum prior to rubbing. If the pipe cleaner is held by the twisted ends and the leaf is supported on a pot label or by a plastic bag covering the hand, the fingers are not contaminated with virus inoculum and the pipe cleaner can be discarded after use.

In tests comparing the efficiency of the pipe cleaner inoculation technique and the conventional cheesecloth pad method, alfalfa mosaic virus from celery (*Apium graveolens* L. var *dulce* (Mill.) Pers.) and Ladino clover (*Trifolium repens* L.) was inoculated on opposite half leaves of Bountiful bean (*Phaseolus vulgaris* L.) seedlings. Comparable numbers of local lesions were produced by each method. Comparable infection also resulted when the 2 methods were compared by inoculating Bountiful bean seedlings with bean yellow mosaic virus; tobacco (*Nicotiana tabacum* L.) and beans with tobacco ringspot virus; and beans and Ladino clover with alfalfa mosaic virus and several unidentified virus isolates.

Any brand of plain, white, cotton pipe cleaner probably is satisfactory. Pipe cleaners generally are available in a package of 36 for 10-15 cents.

A STRAIN OF BARLEY YELLOW DWARF VIRUS TRANSMITTED SPECIFICALLY BY THE CORN LEAF APHID

W. F. Rochow

Department of Plant Pathology, Cornell University, Ithaca, New York.

Plant Pathologist, Crops Research Division, ARS, U.S.D.A.; and Associate Professor, Cornell University.

Cooperative investigation of Crops Research Division, ARS, U.S.D.A.; and Cornell University Agricultural Experiment Station. Supported in part by a PHS research grant, E-2540, from the National Institutes of Health.

Two strains of the barley yellow dwarf virus (BYDV) have been identified on the basis of transmission by *Macrosiphum granarium* (Kby.), the English grain aphid; or *Rhopalosiphum padi* (L.), the oat bird-cherry aphid (formerly referred to as *R. fitchii* (Sand.), the apple grain aphid). One strain is transmitted efficiently by *R. padi;* but rarely, if at all, by *M. granarium.* The other is transmitted efficiently by *M. granarium,* but rarely by *R. padi.* The vector specificity of such strains has been absolute in some tests and relative in others; in all cases, however, clear differences between the 2 virus strains have been shown (1, 5, 6).

It had been suggested (1) that previous failure to recover virus from some oat field samples presumably infected by the BYDV might be explained by infection by another vector-specific strain of the virus, transmissible only by an aphid different from the 2 used in earlier work. Tests in 1959 and 1960, based on use of 4 aphid species, substantiated this possibility. Virus was recovered from a few field samples each year by means of *Rhopalosiphum maidis* (Fitch), the corn leaf aphid; but not by *M. granarium, R. padi,* or *Toxoptera graminum* (Rond.) (2, 3). This paper reports results of tests on the relative vector specificity of 2 such virus isolates, "*R. maidis* virus" (RMV); and on direct comparisons of these isolates with the 2 vector-specific strains previously described. A preliminary report of this work has been given (4).

All colonies of *M. granarium, R. padi,* and *R. maidis* were maintained under the conditions described previously (1). At least 30 aphids from each colony were tested as controls in each experiment; all colonies used were free of the BYDV. The MGV and RPV strains of the virus, previously described as EGV-1 and AGV-1 (1), are transmitted specifically by *M. granarium* and *R. padi,* respectively. The 2 RMV isolates were recovered in 1959 from New York samples of oats (RMV-O) or barley (RMV-B) specifically by *R. maidis* (2). Acquisition feedings of 1–2 days were on detached portions of 1 leaf or on portions of 2 adjacent leaves incubated at 15°C (1, 2). Acquisition feedings of 3–5 days were on intact plants caged in the greenhouse. Inoculation test feeding, at the rate of about 10 aphids/plant on caged Coast Black or California Red oat seedlings, was for 3–5 days in the greenhouse. At least 3 test plants were infested with aphids of each species in any single transmission experiment.

The specificity of each of the 4 virus isolates was absolute when acquisition feedings of 1–2 days were used in serial transmission tests made during 2 years (Table 1). Neither of the RMV isolates was transmitted as readily by *R. maidis* as were the other 2 isolates by their respective vectors. In fact, no transmission occurred in 8 of the 25 tests involved in the 11 serial transmissions of RMV-O. In contrast, RPV and MGV were transmitted in all 25 tests involved in the 13 serial transmissions.

When acquisition feedings of 3–5 days on intact plants were used, specificity of the 4 isolates was

TABLE 1. Transmission of 4 isolates of barley yellow dwarf virus by *Macrosiphum granarium* (MG), *Rhopalosiphum padi* (RP), and *R. maidis* (RM)

Virus isolate[a]	No. of plants infected[b] in tests with aphid species indicated, during no. of serial transmissions shown, following acquisition feedings of 1-2 days on comparable portions of a detached leaf				No. of plants infected[b] in tests with aphid species indicated following acquisition feedings of 3-5 days on intact plants		
	No. of serial transmissions	MG	RP	RM	MG	RP	RM
MGV	13	102/111	0/72	0/72	42/42	2/27	0/21
RPV	13	0/78	116/117	0/78	2/42	44/44	1/42
RMV-O	11	0/72	0/72	48/120	1/24	5/33	19/31
RMV-B	8	0/45	0/45	28/51	0/18	5/30	4/6

[a] MGV, RPV, and RMV isolates were originally transmitted from naturally infected oats only by MG, RP, or RM, respectively. One RMV isolate came from oats (O) and another from barley (B).

[b] Numerator is number of oat test seedlings that became infected; denominator is number of seedlings that were infested with about 10 aphids each for an inoculation test feeding of at least 3 days.

marked; but not absolute (Table 1). Occasional transmission of MGV by *R. padi* and of RPV by *M. granarium* was similar to the relative specificity of these virus strains in previous tests (1). Occasional transmission of RMV occurred more often with *R. padi* than with *M. granarium*, but neither of these species transmitted RMV as readily as did *R. maidis* (Table 1).

A leaf from 11 of the 16 plants infected by means of the "nonvector" in these occasional transmissions was tested by means of the 3 aphid species to determine whether the virus occasionally transmitted by a "nonvector" was the specific isolate in question or a mutant or other selection of virus from it. As in previous tests with MGV and RPV (1), no evidence was obtained in any of the 6 cases to indicate virus selection by the "nonvector." In all tests, the strain involved in an occasional transmission by the "nonvector" apparently was the original strain. For example, when the 1 plant infected by means of *R. maidis* in tests with RPV was tested, BYDV was recovered by *R. padi*, but not by *M. granarium* or *R. maidis*. Such data also provide evidence that the occasional transmissions by a "nonvector" are not accidental contaminations.

These results are considered evidence for the occurrence of at least 3 vector-specific strains of the BYDV in the field; for the relative, not absolute, nature of the vector specificity; and for the stability of the specificity during transfers of the virus strains in the greenhouse.

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THE PRESIDENT'S COLUMN

Although our Society is now in its fifty-third year, it has not had any continuing plan for meritorious awards in recognition of achievement by its members or by plant pathologists in general. As early as 1921, however, it instigated the Eunice R. Oberly Memorial Prize for the stimulation and recognition of excellence in bibliographical work. Miss Oberly had been the librarian for the Bureau of Plant Industry in the U.S. Department of Agriculture. About a thousand dollars was raised by member contribution for this prize. In 1924, the first award was made to Max Meisel, formerly of the Science Division of the New York Public Library, for his extensive bibliography on American Natural History. There is no further report on the Oberly Prize which presumably was not renewed.

Again in 1941, a temporary committee on Recognition of Merit was appointed to study a method of selecting the outstanding paper presented at the annual meeting of the Society. The committee appreciated the difficulty of appraising papers on short notice and among other recommendations suggested a continuing committee composed of the last 5 past presidents. But 3 years later, this committee, apparently unable to achieve a workable method of recognizing meritorious papers, was dissolved.

For at least a third time, the question of awards was brought up informally in connection with the planning of the Golden Jubilee celebration of the Society. The idea, however, was not developed and the Society as a whole was probably unaware of it.

Why bring up this subject again? Various reasons can be given. The rewarding of merit hardly needs justification. Here is an opportunity to honor our peers for valuable and important contributions to the science of plant pathology and in turn the general welfare of mankind. It must be granted that the identification of merit is not simple; and it can become arbitrary, subjective, and even political in nature. The distinction between potential candidates can be very thin indeed. But should we avoid a worthwhile effort because it is difficult? Nowadays, the advantages of bringing science to the general public are pretty well accepted. The honoring of our scientists has newsworthy appeal. Finally, it must be admitted that one of our daughters, the Northeastern Division, is already ahead of us. By the time this column appears in print, they probably will have announced their plan for the recognition of merit on a Divisional basis. Must the parent lag behind a mere youngster?

What is being done by some of the other scientific societies, especially those in which plant pathologists have a particular interest? The American Society of Plant Physiologists has long conferred the annual Charles Reid Barnes Life Membership and the Stephen Hales Award. The Botanical Society of America for many years gave no awards. In commemoration of their Fiftieth Anniversary in 1956, they conferred Golden Jubilee awards on 50 distinguished North American scientists in the botanical field. Awards to several eminent botanists are being continued annually. The Botanical Society also grants the special Darbaker Prize for meritorious work on the algae. The American Society for Horticultural Science gives several awards for the best research paper published during the previous year in various fields of horticultural science. The Potato Association of America awards annually 3 Honorary Life Memberships. The Entomological Society of America similarly confers honorary life membership. One society, the Ecological Society, gives an Eminent Ecologist Citation which is limited to outstanding ecologists who have not yet been honored by the presidency of the Society. We wonder how the time factor is handled! With some of the above awards, there is also a monetary gift. The gigantic American Chemical Society annually grants the famed Priestly Medal and some 25 other awards in various fields of chemistry. Most of these are sponsored by some industrial company and a cash award, usually of $1,000, also is given. This large number of awards does not include those administered by the Sections of the American Chemical Society; for example, the Charles F. Spencer award for outstanding achievement in agricultural chemistry, which is administered by the Kansas City Section.

In general, there apparently are 5 different types of honorary awards: Fellows of a society, awarded on a rather broad basis as in the A.A.A.S., or on a more restricted basis as in the New York Academy of Sciences; honorary life memberships; medals and citations (with or without monetary awards), given for distinguished contributions in a given field; prizes for outstanding papers presented at an annual meeting or in a given year; and more rarely, fellowship awards in cash to students, as with the Mycological Society of America. We believe that our Society might well reopen the question of meritorious awards and adopt one of the above plans.

As a corollary to awards and public recognition, should not our Society have a seal or emblem? At one time, 1935, a committee was appointed to look into this; but nothing apparently came of its deliberations. We have had the unofficial emblem of an Etruscan ring inclosing an *Uncinula* ascus on the cover of PHYTOPATHOLOGY. This was introduced in 1941 during the tenure of Editor-in-Chief H. B. Humphrey; and discontinued a decade later. A rather general specification for a seal could be decided by an appropriate committee or the Council. The more artistic members of the Society then could be asked to submit competitive designs, perhaps even for a special prize!

S. E. A. McC.

WRITING FOR PHYTOPATHOLOGY

Reviewing, editing, and publication of articles will be facilitated if procedures outlined below are followed. For style details not mentioned here, follow Style Manual for Biological Journals (1).

Manuscripts must be typed *double spaced throughout*, preferably on a good grade of 8.5 x 11.0 in. white paper. Authors are urged to have manuscripts reviewed critically by one or more colleagues.

Subject matter must be: 1) unpublished original research; 2) review papers developing a new concept, hypothesis, theory, or other integration of plant pathology. Submission implies nonsubmission elsewhere and (if accepted), no publication elsewhere in the same form without consent.

TITLE.—Short, informative titles are desirable. Stating the factors investigated is much better than merely, "studies of." Type titles in solid capitals. Do not underline scientific names and omit authorities for scientific names in titles.

The department and institution sponsoring the work is now appearing on the first line below the author's name. Other credits, titlenotes, and authornotes follow this. The acceptance date appears as the last line before the Summary.

SUMMARY

The "Summary" should inform a reader of the scope of the article and its significant findings. Give the facts; do not refer to them as "are described." The "Summary" should be about 3% as long as the article and complete enough for use in Biological Abstracts. "SUMMARY" should be completely capitalized and centered on the page. Use a solid, typed line to separate "Summary" from "Introduction."

INTRODUCTION.—State the problem briefly and the reason for presenting the results. If a "Review of Literature" is used to present the problem, it may be part of the "Introduction." This and subsequent section headings (except "Literature Cited") should be completely capitalized and indented as part of a paragraph.

REVIEW OF LITERATURE.—If necessary as background for the article, related, pertinent literature should be summarized.

MATERIALS AND METHODS.—Materials and techniques used throughout the work should be described once, in this section. Detailed procedures of individual experiments and trials are best deferred to the "Results" section.

RESULTS.—Present results in logical rather than chronological order. Do not interpret the obvious, but statements of why experiments were done aid in transitions from one topic to the next. Subheadings are desirable as reading aids; indicate them as follows:

RESULTS.—

Second subhead.—

1) *Third subhead.*—

a) *Fourth subhead.*—

Do not use footnotes. Use arabic numerals for all numbers, except in titles and at the beginning of a sentence. Centigrade temperature and scientific rather than common names of organisms are preferred.

DISCUSSION.—This section should *not* be a summary. Results should be summarized here only as necessary for discussing their implications and for relating them to other problems or other results on the subject.

LITERATURE CITED

1. Committee on Form and Style, Conference of Biological Editors. 1960. Style manual for biological journals. Am. Inst. Biol. Sci., Washington, D.C. 92 p.
2. PRICE, W. C. 1954. Preparing manuscripts for Phytopathology. Phytopathology 44:667-674.
3. RIKER, A. J. 1946. The preparation of manuscripts for Phytopathology. Phytopathology 36:953-977.

All citations should be numbered consecutively under the above heading. Arrange alphabetically and chronologically under one or the same authors. Use only initials for given names of male authors, but spell out first names of women. For periodical abbreviations not given in the AIBS Style Manual, follow Chemical Abstracts "List of Periodicals." Unpublished data, personal correspondence, *etc.*, should not be cited as literature, but referred to in parentheses in the text. Authors' names should be in capital letters (except "organizations" as in 1. above). Type number flush with the left margin; indent all succeeding lines 5 spaces.

Order of manuscript.—Literature citations should be followed by: tables; figure captions; illustrations. Each sheet should be numbered consecutively (except illustrations) and should bear the authors' names in the upper right hand corner.

Tables.—Titles of tables should indicate clearly the contents, so the table will be understandable without reference to the text. Present data in a table *only* when they cannot be presented clearly in the text. Do not use vertical lines in tables. Indicate footnotes with lower case superscript letters. Abbreviate wherever possible; follow the AIBS Style Manual.

Illustrations.—Photographs must be good quality, high contrast prints on glossy paper. Whenever practical, combine illustrations and identify each component by a letter. Composite illustrations must be rectangular or square and proportioned to fit a PHYTOPATHOLOGY page. For example, if the vertical dimension of a full-page illustration is more than 1.25 times the width, there will be insufficient space beneath for the caption. Captions for illustrations may be typed on a single sheet. Do not combine them with illustrations. If cost of illustrations exceeds the cost of 1 full-page cut, authors will be charged for half the excess.

Graphs, charts, and line drawings may be submitted as originals or as photographic reproductions. The identity of multiple lines on graphs should be

indicated by arrows or a legend on the graph, rather than in the caption. Authors will be charged for graphs and line drawings exceeding 30% of the length of the article.

Dimensions of illustrative material submitted should be the same as text sheets. Oversize illustrations are easily damaged in the mail and impossible to file with the manuscript. Illustrations should be submitted in duplicate, mounted on heavy cardboard, each identified with authors' names, title of article, and figure number. Protect illustrations with heavy cardboard in mailing.

Phytopathological notes.—The above instructions apply equally to "Notes," except that "Notes" must be sufficiently brief to need no summary. They may deal with research or matters of historical or current interest. Routine or trivial subjects are no more desirable for "Notes" than for articles. The editors will endeavor to publish "Notes" as soon as possible after acceptance.

Letters-to-the-editor.—These may be concise statements of opinion on plant pathological subjects, announcements, or criticism. They will be reviewed as are other types of manuscripts and the editorial board reserves the right to reject letters or to publish rebuttals when appropriate.

Submissions.—Send mss and correspondence to the Editor-in-chief. At least 1 author must be a member of The American Phytopathological Society, except that each subscribing institution outside the U.S. and Canada is allowed 1 published paper by a staff member in each volume of PHYTOPATHOLOGY. A letter of transmittal, signed by an official of the foreign institution, should accompany such manuscripts. English is required.

Chemical nomenclature.—Trade or code names must be followed by chemical names of active ingredients, with any percentages less than 100%. (For chemical names of fungicides, see Phytopathology 45:295-302.) Structural formulas included in the text should be submitted separately as drawings.

Grammar.—Follow patterns suggested in Style Manual for Biological Journals.

Capitals.—Generally, only titles, cited authors, and major headings.

Underlining.—Generally, only words that should be italicized.

Editorial procedure and policy.—Normally, manuscripts are sent by the editor to a reviewer, usually a member of the editorial board. Reviewers may consult with such other authorities as necessary to confirm the scientific merit of any part or all of the manuscript. In event of an unfavorable review, manuscripts are sent to a second and sometimes a third reviewer for additional opinions.

After review, manuscripts are edited for grammar and conciseness. Changes may be suggested to achieve uniformity in style, clarity of presentation, and economy of words.

NEWS

Editor: K. W. Kreitlow, Plant Industry Station, Beltsville, Md.

The Rockefeller Foundation elected phytopathologist J. G. Harrar President to succeed Dean Rusk, who resigned to become Secretary of State. Dr. Harrar was formerly Director of Agriculture and a Vice President of the Foundation since 1959.

Plant pathologist Ruben Duran of the USDA Marketing Research Division Laboratories, located at Pomona, California, has accepted a position at Washington State University, Pullman.

Phytopathologist Raymond Clark a recent graduate of Washington State University, has accepted a position with the USDA. He will investigate curly top resistance in tomatoes at the Irrigation Experiment Station, Prosser, Washington.

Acting Chairman C. Gardner Shaw, of the Department of Plant Pathology, Washington State University, Pullman, has changed status to elected Chairman for the next 4 years.

Pathologist J. C. Carter, Head of the botany and plant pathology section of the Illinois Natural History Survey, who is president of the National Shade Tree Conference, presided at the 37th annual convention of the organization in Minneapolis August 20-25.

Forest pathologist Frances A. Wood, a recent graduate of the University of Minnesota, has assumed teaching and research duties as Assistant Professor of forest pathology at Pennsylvania State University, University Park, Pennsylvania.

Plant pathologist B. W. Kennedy has been recently appointed a Research Associate in the Department of Plant Pathology and Botany at the University of Minnesota. Dr. Kennedy, a graduate of the University of Minnesota, will study diseases of soybeans.

Graduate courses in plant pathology are being offered for the first time at the Riverside campus of the University of California. Instruction will be given by the faculty in the Citrus Experiment Station. Those interested in information on the new master's or doctor's degree program should write to the Department of Plant Pathology at Riverside.

Pathologist David E. Zimmer, Oilseed and Industrial Crops Research Branch, USDA has been appointed to conduct research on diseases of safflower at Logan, Utah.

Phytopathologist Lawrence A. Schaal recently accepted the assignment as superintendent of the Horticultural Field Station at Cheyenne, Wyoming. In this capacity, he is in charge of all local arrangements for the vegetable, small fruit, windbreak, and other horticultural research at that Station.

Visiting plant pathologist Alberto Matta of Italy, holder of a NATO science fellowship, is at the Connecticut Agricultural Experiment Station for a six-month period to work with station scientists. Dr. Matta, who is affiliated with the Universiy of Turin, will study wilt diseases of tomato and their control by chemotherapy, with special emphasis on *Verticillium* wilt.

Phytopathologist William C. Snyder, Vice Chairman of the Department of Plant Pathology at the University of California, has been invited to the Waite Agricultural Institute at the University of Adelaide in Australia for consultations on root diseases and Fusarium fungus problems. Dr. Snyder plans to attend the Pacific Science Congress in Hawaii enroute to Australia. During his stay in Australia, he also will present two papers at the Australian Plant Pathology Conference. The papers will deal with studies being conducted at the University of California on Fusarium and biological control of root diseases. He will visit universities in Japan on his return journey to the United States to exchange information with other plant pathologists on Fusarium research problems. The trip is being made under the auspices of the Rockefeller Foundation.

An invitation paper entitled "Host-parasite relationships in plant disease" was presented by phytopathologist H. H. Flor at the Gordon Research Conferences held at Tilton, New Hampshire, July 24 to 28.

Plant pathologist Duane L. Coyier, Fruit and Nut Crops Research Branch, USDA, has been appointed to conduct basic and applied research on apple and pear fungus and bacterial diseases at Hood River, Oregon. He recently completed his Ph.D. in plant pathology at the University of Wisconsin, Madison.

Pathologist William M. Dowler who recently completed his Ph.D. degree in plant pathology at the University of Illinois, Urbana, has been appointed to work with D. H. Petersen on crown rot and bacterial canker affecting peach trees at Clemson, South Carolina.

A prize of I.E. 500 (approximately $1000.00) has been awarded by the Government of the United Arab Republic to phytopathologist Farid Nour-Eldin of the Egyptian Ministry of Agriculture for outstanding contributions to the country's agriculture. Of 87 scientists submitting 560 papers, Dr. Nour-Eldin was chosen one of 16 winners on the basis of the best papers published during the period 1958-1960. Dr. Nour-Eldin is Head of the Virology Department of the Section of Plant Pathology and a specialist in viruses affecting citrus.

Phytopathologist John M. Klisiewicz, Oilseed and Industrial Crops Research Branch, USDA, has been appointed to conduct research on diseases of safflower and castorbeans at Davis, California. Dr. Klisiewicz replaces Kenneth E. Mueller, who resigned to become Superintendent of the Rice Experiment Station at Biggs, California.

Emeritus professor Peter A. Ark, University of California, Berkeley, was honored along with Mrs. Ark at a dinner on August 26. Approximately 100 friends, colleagues and students attended. Professor Ark will continue his research on bacterial diseases of plants at Berkeley.

Cotton diseases will be investigated by J. A. Pinckard, Jr., who recently joined the Louisiana State University as an Associate Professor of Plant Pathology. Dr. Pinckard was formerly associated with the Shell Chemical Company.

Pathologist Gerald S. Walton, who recently completed his graduate studies at Rutgers University, New Brunswick, New Jersey, has joined the staff of the Department of Botany and Plant Pathology of the Connecticut Agricultural Experiment Station, New Haven. Dr. Walton replaces Mr. Ernest M. Stoddard.

Plant pathologists Floyd F. Hendrix, Jr., and E. George Kuhlman have joined the staff of the Division of Forest Disease Research, Southeastern Forest Experiment Station, U. S. Forest Service at Asheville, North Carolina. Dr. Hendrix, a 1961 graduate of the University of California, and Dr. Kuhlman, a 1961 graduate of Oregon State University, have both been assigned to a research team studying root rots and other diseases of forest trees in the Southeast.

Pathologist Arthur M. Elliot has accepted a position with the Biology Department of Texas Technological College, Lubbock, Texas, as an Assistant Professor. Dr. Elliot will teach and conduct research.